THE AVAILABILITY OF ORGANIC NITRATES FROM INTRAVENOUS ADMINISTRATION SYSTEMS

by

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SUMMARY

Nitroglycerin and isosorbide dinitrate are two drugs which are infused intravenously during the treatment of ischaemic heart disease. The availability of these two drugs in solutions infused from plastic infusion bags or glass infusion bottles through plastic giving sets has been investigated.

During simulated infusions the concentration of nitroglycerin and isosorbide dinitrate appearing in the effluent of the giving set tubing was found to be much less than the concentration of the drug solution initially contained in the plastic infusion bag or glass infusion bottle. It was found that each component of the plastic infusion equipment sorbed the drugs to a significant extent and that the rate of disappearance of drugs from solutions stored in each component was in the rank order: giving set tubing > giving set burette > plastic infusion bag. There was no significant loss of either drug from solutions stored in glass bottles. The influence of formulation factors and storage conditions on the sorption of nitroglycerin, isosorbide dinitrate and another organic nitrate compound, ethylene glycol dinitrate, by plastic infusion equipment was studied. The extent of loss during simulated infusions was also found to be dependent on flow rate of drug solution through the giving set.

The sorption of nitroglycerin and isosorbide dinitrate has clinical and pharmacokinetic significance.

Losses of nitroglycerin and isosorbide dinitrate associated with their infusion through plastic infusion equipment were minimised by infusing drug solutions from a glass syringe through high density polyethylene tubing. This method was also successfully applied to overcome the previously reported loss of diazepam and chlormethiazole during infusions using conventional plastic administration equipment.

CONTENTS

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University.

To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

(P.A.Cossum)

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CHAPTER 1

INTRODUCTION

1.1. INTRAVENOUS ADMINISTRATION OF ORGANIC NITRATES

Intravenous (I.V.) medication is administered directly into a vein either to provide an initial rapid response which may be maintained with further infusion of drug, or to overcome the variations in bioavailability associated with other routes of administration. This route of administration ensures maximum bioavailability. The duration of drug activity is dependent on the initial dose and the pharmacokinetics of the drug. Drugs with short half-lives and for which constant and sustained drug amounts are required, are often administered as a continuous I.V. infusion (Boylan and Fites, 1979; Niazi, 1979). The extent and rate of drug delivery with I.V. infusions are generally better controlled than other methods used to obtain sustained drug action.

A number of studies have shown that the organic nitrates can be administered intravenously for specific therapeutic applications. From experiments in dogs Epstein et al (1975) found that when nitroglycerin (GTN) was administered intravenously as a 400mcg bolus followed by a continuous infusion of 300 mcg/min, coronary occlusion produced much less ischaemic injury than it did in dogs not previously

treated with nitroglycerin. These workers concluded that it is possible that the administration of nitroglycerin to patients suffering acute myocardial infarction, irrespective of the presence of left ventricular failure, would reduce the frequency and severity of transmural infarction and so enhance the prospects of long term survival.

Chiche et al (1979) showed that prolonged nitroglycerin infusion resulted in less progression of electrocardiographic parameters of ischaemia and necrosis in patients suffering acute myocardial infarction.

Intravenous infusions of isosorbide dinitrate (ISDN) have been used successfully for the treatment of severe angina (Distante et al, 1979).

In late 1977 some patients admitted to the Royal Hobart Hospital suffering acute myocardial infarction failed to respond adequately to nitroglycerin given by intravenous infusion. Little haemodynamic response was observed except at the initial infusion rate, despite a continual increase of nitroglycerin infusion rate (to a final intended value of 300 mcg/min). Two possibilities were suggested to explain the poor response of those patients; either nitroglycerin was not being delivered to the patients in sufficient quantity due to interaction with the delivery system, or the patients failed to respond to the drug as a

result of some pharmacodynamic/pharmacokinetic factor. This present work reports the studies resulting from investigation of the former possibility.

1.2. DEVELOPMENT OF PLASTIC DELIVERY SYSTEMS

1.2.1. Intravenous Infusion Bags.

The polyvinyl chloride intravenous fluid bag (Viaflex) was introduced by Travenol Laboratories in 1971. These bags have some advantages over glass infusion bottles and are therefore preferable for certain therapeutic indications. Because plastic infusion bags collapse on emptying, the need to replace an equivalent volume of air is overcome and so the risk of contamination from air-borne microbes is greatly reduced, as is the risk of embolism. Furthermore, extra medication can be added by syringe to the plastic infusion bag without the risk of air entering the bag. In order to pressure-infuse solutions, the soft bag can be squeezed. Other lesser points of advantage include the bag being easy to support in the correct position, its resistance to breakage and the ease of disposal of used bags.

Petrick et al (1977) have reviewed the problems associated with the use of plastic intravenous bags. The major problems which arise in using these bags include human touch contamination, the generation of particulate matter,

the potential to insufficiently mix I.V. additives when injected into plastic I.V. bags, the leaching of toxic stabilizers and/or plasticizers from the plastic into the I.V. solution and sorption of drugs from solution by the plastic I.V. bag. Petrick et al (1977) concluded that only a financial consideration made the use of the plastic I.V. bag more advantageous than the glass bottle.

1.2.2. Intravenous Infusion Giving Sets

The method of administration of intravenous fluids had for many years been through plastic tubing with only a crude means of controlling the flow rate. In the early 1970's several companies released a plastic volume control set which offers several distinct advantages over those administration sets previously used. The in-line burette, made of cellulose propionate, allows accurate measurement of fluid intake and at the same time limits the volume of fluid which may be accidentally infused. The burette chamber also offers the provision of intermittent intravenous therapy with different drugs, and a membrane filter at the base of the burette prevents any air or microbial contaminants being infused into the patient.

Henry and Harrison (1972) discussed a number of potential errors which could occur with the use of plastic volume control giving sets. These ranged from accidental flooding of large volumes of intravenous fluid, to the admixture of

two or more incompatible drugs in the burette. Duma et al (1971) found that the intravenous giving set was frequently a starting point for bacterial infections in the patient being infused and the contamination arose from the hands of personnel changing the giving set during the introduction of additives. Henry and Harrison (1972) concluded that contamination is compounded each time medication addition ports are used. These ports, invariably set upright, can become gathering points for dust and other contamination and can lead to infection unless regularly cleaned before use. Contamination of volume control sets by Bacillus subtilus, Staphylococcus epidermidis and Aspergillus spp was reported by McAllister et al (1974).

The variation in drip rate within gravity-fed plastic giving sets can result in a very large fluctuation in volume being delivered to a patient (Flack and Whyte, 1974). This has been shown to be almost completely due to variation in the patient's venous pressure which changes dramatically with posture. Flack and Whyte (1974) recommended that to obtain a relatively constant flow rate, gravity-fed giving sets should be provided with a servo-control mechanism.

1.3. ORGANIC NITRATES

1.3.1. Pharmacology

Although nitroglycerin and isosorbide dinitrate are the most widely used organic nitrates, the pharmacology of erythritol tetranitrate, pentaerythritol tetranitrate and mannitol hexanitrate has also been evaluated (Krantz et al, 1939a; Carr, 1975).

The basic pharmacological action of the organic nitrates is to relax smooth muscle resulting in an increase in venous capacitance. In this way elevated ventricular filling pressure, central venous pressure and end-diastolic volume are reduced and the overall result can be a reduction in the myocardial oxygen demand (Brachfeld et al, 1959; Rowe et al, 1961; Parratt, 1980). Nitroglycerin has also been shown to dilate arteriosclerotic coronary vessels (Parratt, 1980). Through their effects on smooth muscle, organic nitrates also reduce pulmonary oedema and have been used to treat biliary colic.

Nitroglycerin has been shown to possess moderate positive inotropic activity (Parratt, 1979) and to decrease the susceptibility to ventricular fibrillation during acute myocardial ischaemia (Stockman et al, 1979).

Reviews of the pharmacological action of organic nitrates on smooth muscle have been presented by Carr (1975), Needleman and Johnson (1975) and Parratt (1980).

Nitroglycerin and isosorbide dinitrate have also been shown to be inhibitors of platelet function (Schafer et al, 1980).

1.3.2. Therapeutics

Continuous intravenous infusions of nitroglycerin have been used in the therapy of acute myocardial infarction (Flaherty et al, 1975; Armstrong et al, 1975; Come et al, 1975). It is suggested that nitroglycerin reduces ischaemic injury to the myocardium when administered immediately after the onset of a myocardial infarction, thus limiting infarct size. Nitroglycerin infusions are also used to decrease cardiac pre- and after-loads in patients undergoing coronary artery bypass surgery (Kaplan et al, 1976; Glisson et al, 1980).

Distante et al (1979) have shown that a continuous intravenous infusion of isosorbide dinitrate is an effective treatment for patients with vasospastic angina at rest.

CHAPTER 2

AVAILABILITY OF DRUGS FROM PARENTERAL SOLUTIONS

2.1. DRUG-DELIVERY SYSTEM INTERACTIONS

2.1.1. Types of Interactions

Drugs and other additives in parenteral solutions may interact with delivery systems in numerous ways.

(a) Adsorption

Adsorption describes the interaction at the interface of a drug solution-delivery system. Adsorption occurs in both glass and plastic containers and results in a reduction of the drug concentration in the infusion solution.

(b) Absorption

The partitioning and diffusion of a drug into the matrix of a membrane or container wall is referred to as absorption. This process does not readily occur in glass containers.

Adsorption and absorption together comprise the process of sorption. The partition coefficient of a solute between its aqueous solutions and a plastic system is the major factor determining whether sorption will occur (Jordan and

Polack, 1972) and this may be dependent upon the pH of the solution and the nature of the solvent system. Temperature, the concentration of solute and ionic strength of the solution are other factors which may increase or decrease the magnitude of sorption (Autian, 1971).

(c) Permeation

Permeation is the movement of solute molecules from the environment on one side of a membrane to the environment on the other side. Sorption is often an essential prerequisite for permeation since permeation usually cannot occur unless the solute has been sorbed by the plastic.

The nature of the penetrant molecule as well as the properties of the plastic material are the major factors influencing the rate of transfer of molecules from one side of a plastic membrane to the other side. Temperature, pH, nature of the solvent, the concentration of the solute and the ionic strength of the solution all influence the sorption and permeation of molecules from their aqueous solution into/through a plastic sheet (F.D.A. notes, 1974/75).

(d) Leaching

Leaching refers to the migration of an ingredient of a plastic or glass material into a solution in contact with

the container. Compounds leached from plastic containers are generally plasticizers, stabilizers or antioxidants. Leaching of silica, alumina and alkali can occur from glass (Autian, 1971).

Leaching is influenced by the solubility of leached material in solution, temperature, excessive agitation of the filled container, pH and time of contact of solution with container (Autian, 1971).

(e) Chemical Reactivity

The interaction of a polymer or one of the additives in the polymer with a drug can occur. Very rarely does the chemical reaction of a diffusing molecule occur with the polymer itself. More often it occurs with an antioxidant in the plastic. Staining or discolouration of a plastic is the most common result of the chemical reaction of a parenteral additive and a plastic (Autian, 1971).

(f) Alterations in the Properties of a Container

Sorption, permeation, leaching and chemical reactivity may all result in the physical condition of a plastic container being changed. A sorbed ingredient may act as a plasticizing agent and permit greater polymer chain flexibility which increases the permeation of sorbed molecules or gases through the plastic. When an

antioxidant is leached from a plastic, oxidation of the polymer chains occurs with subsequent degradation of the polymer (Autian, 1971).

Degradation of some types of polymers can also occur under the influence of heat and ultraviolet light.

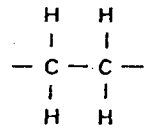
2.1.2. Models of Drug Sorption Interactions

(a) Partition Coefficient and the Two Compartment Model

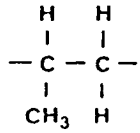
In considering the sorption of a drug from its aqueous solution by a plastic, the partition coefficient is expressed in terms of the ratio of the equilibrium concentration of the drug in the non-aqueous phase to the concentration in the aqueous phase. The plastic-water partition coefficient may be viewed as an index of the relative affinity of the drug for a plastic and the vehicle in which the drug is dissolved.

The partitioning of a drug between the aqueous phase and a plastic, such as polyvinyl chloride or polyethylene, may be considered analagous to the partitioning of a solute between water and oil. Moreover, aliphatic hydrocarbons can be considered to have similar partitioning characteristics to polyvinyl chloride and polyethylene because like those two plastics, aliphatic hydrocarbons such as (n-)hexane and (n-)octanol have free $-CH_2-$

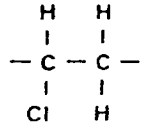
POLYETHYLENE



POLYPROPYLENE



POLYVINYL CHLORIDE



CELLULOSE PROPIONATE

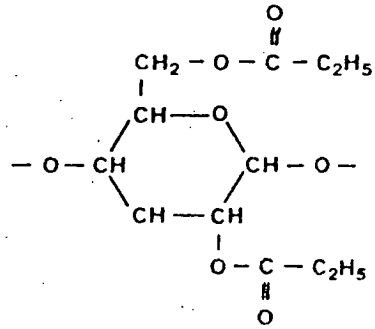


Figure 2.1 Unit structures of some polymers used in medical applications.

functional groups (Figure 2.1)

Polack et al (1970) related the loss of a number of solutes from polyethylene containers to the hexane-water partition coefficient of the solute. Further work by Jordan and Polack (1972) and Roberts et al (1979) confirmed that the rate of permeation of a solute through polyethylene films may be correlated with the hexane-water partition coefficient of the solute.

From a study of eleven binary systems, Smith et al (1975) suggested that the octanol-water system is the best reference system for biological partitioning and activity in drug design work. It is possible that octanol-water partition coefficients may also be used to predict the extent of uptake of drugs by polymer films. Octanol-water partition coefficients have been correlated by Kowaluk et al (1981) and Krieglstein et al (1972) with the extent of sorption of a series of phenothiazines by polyvinyl chloride.

The disappearance kinetics of some solutes from solutions stored in plastic containers has been described by a two compartment open model (Roberts et al, 1979; Polack et al, 1979) (Figure 2.2).

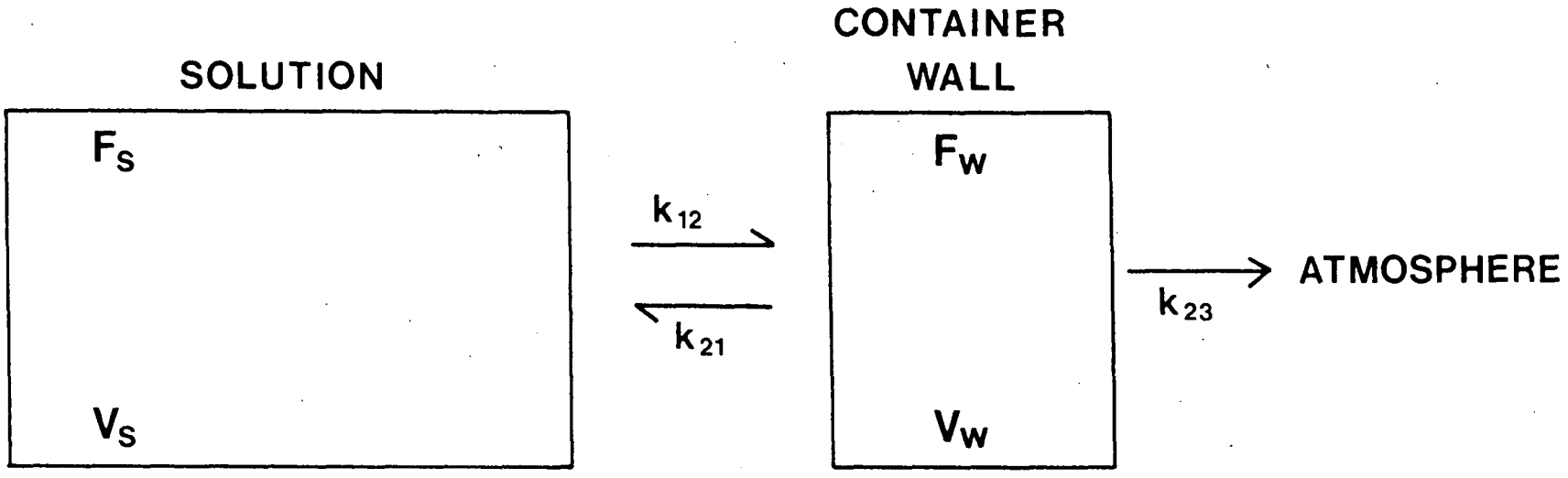


Figure 2.2 Two compartment model used to examine disappearance kinetics of solutes from aqueous solutions stored in plastic containers. 'F' and 'V' refer to the fraction of solute remaining in, and the volume of, each of the compartments 'S' and 'W', the solution and container wall respectively. (See text for details).

This model describes the uptake of solute from its solution by the walls of a container followed by a slower and essentially irreversible loss of the solute to the atmosphere in a bi-exponential manner described by the equation:

$$F_s = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{eqn 2.1}$$

where F_s is the fraction of solute in solution, A and B are the fractional zero intercepts (A+B=1) and α and β are the faster and slower rate constants of disappearance.

Monoexponential disappearance kinetics (equation 2.2) apply if the solute has only a low affinity for the material of the container and an insignificant amount of solute is therefore sorbed by the container wall:

$$F_s = Ae^{-kt} \qquad \text{eqn 2.2}$$

where k is the disappearance rate constant.

The two compartment closed model (equation 2.3) describes a reversible first-order process. The amount of drug remaining in solution at any time is given by the equation:

$$\ln (A_t - A_{eq}) = \ln (A_o - A_{eq}) - (k_{12} + k_{21})t \qquad \text{eqn 2.3}$$

where A_o, A_{eq}, and A_t are solution concentrations at zero, equilibrium and any time 't' respectively, and k_{12} and k_{21} are rate constants of the forward and reverse reactions.

Processes involving loss of a drug from solutions stored in plastic containers and which can be described by a two compartment model are assumed to be essentially partitioning processes. That is, the rate and extent of disappearance of solutes from solutions stored in plastic containers are determined primarily by the transfer of solute across the solution-plastic interface. It is assumed that "mixing" of the solute with the plastic container is instantaneous and complete so that transport within the plastic matrix does not constitute a rate-limiting barrier.

(b) Diffusion Coefficient and the Diffusion Model

Diffusion is the process by which matter is transported from one part of a system to another as a result of random molecular motions. The quantitative description of this process has been summarised in Fick's first law of

diffusion:

$$q = -DA \frac{dc}{dx} \qquad \text{eqn 2.4}$$

where q is the amount of material diffusing across a unit area A of a membrane per unit time, dc/dx the concentration gradient across the membrane and D the diffusion coefficient. In drug-plastic interactions involving sorption, the rate-determining step of the sorption process has been described as the diffusion of the drug molecules within the polymer (Autian, 1971). When the concentration (c) of the drug in the plastic is continually changing, Fick's second law (equation 2.5) is used to describe the rate of change of concentration.

$$\frac{dc}{dt} = D \frac{d^2c}{dx^2} \qquad \text{eqn 2.5}$$

A solution to equation 2.5 has been provided by Crank (1948) and Carman and Haul (1954) (equation 2.6) and was derived from equations given to describe the sorption of solutes from a stirred solution of limited volume by a plain sheet:

$$\frac{F_t - F_\infty}{1 - F_\infty} = \Sigma_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-q_n^2 Dt/l^2\right) \qquad \text{eqn 2.6}$$

where α equals $F_\infty /(1-F_\infty)$, q_ns are the non-zero positive roots of tan $q = -\alpha q$, and D is the diffusion coefficient of solute in a plastic sheet of thickness l. It is assumed

that (1) the concentration of solute sorbed by the plastic is proportional to the free concentration of solute, (2) diffusion of solute in the matrix of the plastic is the rate-determining step in the sorption process, (3) the properties of the plastic and the diffusion coefficient of the solute in the plastic remain unaltered during the sorption process, (4) the concentration of solute in the aqueous solution at any time is independent of position, and (5) sorption of solute by the plastic is restricted to the area in contact with solution.

(c) Temperature Studies

In both the diffusion model and the compartmental model the extent of uptake of drugs by and the rate of diffusion of drugs in plastics is temperature dependent. This temperature dependence has been used to evaluate the mechanisms of interactions between solutes and plastics (Autian, 1971). The dependence of the diffusion coefficient (D) on temperature is usually evaluated using the Arrhenius relationship:

$$D = D_o e^{-E_a/RT} \qquad \text{eqn 2.7}$$

where D_o is a pre-exponential factor, E_a the activation energy of diffusion, and R and T are the usual gas and temperature constants. The activation energy from this expression may be related to the movement of the polymer

chains in a particular plastic and the transport of molecules between those chains (Autian, 1971). The changes in the diffusion coefficient with temperature mainly account for the differences in the extent of sorption of any solute by a plastic at a given time.

2.1.3. Delivery Systems

The delivery systems used for parenteral solutions of drugs and which have been implicated in interactions with drugs are glass bottles, plastic syringes, plastic infusion bags and plastic giving sets.

(a) Glass Bottles

Numerous studies have documented the fact that insulin is adsorbed onto glass surfaces. Ferrebee et al (1951) first reported insulin binding by laboratory glassware. However Weisenfeld et al (1968) were the first to study the adsorption of insulin by glass intravenous infusion bottles. These workers found that prewashing the glass bottles with a 1% albumin solution overcame the loss of insulin by adsorption. Subsequent reports (Petty and Cunningham, 1974; Genuth, 1973; Weber et al, 1977) reported varying success using this technique.

(b) Plastic Syringes

Nylon, polyethylene, polypropylene and polystyrene are the plastics used to make syringes. Autian and Brewer (1958) first reported a drug-plastic interaction involving a plastic syringe. Erythromycin in this case interacted with its solvent diethyl carbonate to degrade the syringe. Adrenalin, promethazine, procaine and streptomycin have been shown to discolour polyethylene syringes (Guess et al, 1965).

(c) Plastic Infusion Bags

The sorption of drugs by plastic infusion bags is well documented. Vitamin A acetate, sodium warfarin, and methohexital (Moorhatch and Chiou, 1974a), thiopentone, chlormethiazole and promethazine (Kowaluk et al, 1981), insulin (Petty and Cunningham, 1974; Whalen et al, 1979; Weber et al, 1977), and diazepam (Parker et al, 1979; Parker and MacCara, 1980; Cloyd et al, 1980) are drugs which have been shown to be appreciably sorbed by plastic infusion bags.

Leaching of the plasticizer di-2-ethyl-hexyl phthalate (DEHP) from polyvinyl chloride infusion bags into blood and other solutions stored in the bags has also been reported (Whitlow et al, 1974; Jaeger and Rubin, 1972; Moorhatch and Chiou, 1974b).

(d) Plastic Giving Sets

Like plastic infusion bags, plastic giving sets have been shown to be responsible for the sorption of a number of drugs. Insulin (Weisenfeld et al, 1968; Hirsch et al, 1977; Whalen et al, 1979), diazepam (MacKichan et al, 1979; Cloyd et al, 1980; Parker and MacCara, 1980) and chlormethiazole (Tsuei et al, 1980) are sorbed by plastic giving sets. Cloyd et al (1980) are the only workers to have looked at the individual contributions of the cellulose propionate burette chambers and the polyvinyl chloride tubing of giving sets.

2.2. DRUG INSTABILITY

Drugs in parenteral solution are subject to several chemical degradative processes which can yield new and often therapeutically inactive or toxic by-products. Moreover, drugs may interact with other excipients in the same parenteral solution resulting in reduced availability of active therapeutic agent.

2.2.1. Hydrolysis

Hydrolysis is the most common chemical reaction responsible for drug degradation in parenteral solutions (Newton, 1978). It is catalyzed by hydrogen ion, light, heat and

divalent metal cations.

Functional groups most susceptible to hydrolysis are esters (Anschel et al, 1972; Higuchi et al, 1950; Marcus and Taraszka, 1957; Schmid, 1961; Moffett and Garrett, 1955), amides (Marcus and Taraszka, 1959), lactams (Chatterji et al, 1975; Savello and Shangraw, 1971; Yamana and Tsuji, 1976) and imines (Han et al, 1976, 1977).

2.2.2. Oxidation

After hydrolysis the next most common pathway of drug degradation is oxidation. The most common form of oxidative decomposition is autoxidation through a free radical chain process (Mollica et al, 1978). Oxidation may be catalyzed by heavy metal ions, oxygen, hydrogen ions, hydroxyl ions, light and heat.

Examples of drugs administered parenterally which can be affected by oxidation include the phenolic drugs morphine (Yeh and Lach, 1961) and phenylephrine (Newton, 1978), the catecholamine epinephrine (Hajratwala, 1975), the phenothiazines (Roseboom and Fresen, 1975; Underberg, 1978) and amitriptyline (Enever et al, 1975).

2.2.3. Photolysis

Light can cause photochemical oxidation or hydrolysis of drugs in parenteral solution. Many examples of this have been reported (Newton, 1978; Lin and Lachman, 1969). A photo-induced rearrangement reaction of sodium nitroprusside has also been reported (Frank et al, 1976).

The ultraviolet and violet portions of the light spectrum are more active in initiating chemical reactions than light from the longer wavelength portions of the light spectrum. Because amber glass does not appreciably transmit light of wavelengths below about 500nm, it is used to effectively retard photolysis of drugs stored in glass. However, plastic polymers have functional groups (carbonyl and aromatic) which absorb ultraviolet light. Resulting degradation products such as hydroxyl or peroxide groups may tend to increase the absorption of ultraviolet wavelengths and hence accelerate any photolytic decomposition already occurring in the solution stored in the plastic (F.D.A. notes, 1974/75). For this reason plastic containers which are used to parenterally administer drugs which are susceptible to photolysis are covered with aluminium foil or other light-shielding material.

2.2.4. Drug-Excipient Interactions

Incompatibilities arising from physicochemical phenomena can result in decreased potency of a drug in parenteral solutions. The incompatibility may be manifested as a drug-drug interaction (Parker, 1967; Newton, 1978; Edward, 1967), a drug-vehicle interaction (Savello and Shangraw, 1971; Blaug and Huang, 1973; Laegeler et al, 1974; Parker, 1967), or a drug interaction involving complexation with metal cations (Price et al, 1957; Prasad et al, 1974) or disodium EDTA (Kirschenbaum and Latiolais, 1976). Interactions between drugs and antibacterial preservatives (Shoup, 1967) or antioxidants (Rork and Pitman, 1975) also occur.

Griffin and D'Arcy (1975) have presented a comprehensive list of incompatibilities of a number of classes of infusion fluids with additives as well as some common drug-drug interactions.

2.2.5. Other Degradative Pathways

Other chemical reactions responsible for the degradation of drugs in parenteral solution are isomerization of penicillins (Bundgaard, 1971), lincomycin monoesters (Oesterling and Metzler, 1972) and prostaglandins E_1 and E_2 (Monkhouse et al, 1973) and epimerization of tetracyclines (Schlecht and Frank, 1973) and l-adrenalin (Hellberg, 1955).

CHAPTER 3

CHEMISTRY OF THE ORGANIC NITRATES

3.1. SYNTHESES

3.1.1. Nitroglycerin

Nitroglycerin (1,2,3-propanetriol trinitrate, glycerol trinitrate, glonoin), which was first synthesized by Sobrero in 1847 (Urbanski, 1965), is a colourless oil which is prepared by the nitration of anhydrous glycerol with equal volumes of concentrated nitric and sulphuric acids (Boschan, 1955; Urbanski, 1965). Sulphuric acid is present because of its hygroscopic nature which prevents water formed in the reaction of glycerol and nitric acid from diluting the reaction mixture. Dilution of nitric acid leads to the formation of the dinitroglycerols (Urbanski, 1965).

3.1.2. Isosorbide Dinitrate

Isosorbide dinitrate (1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate) is a colourless crystalline solid which is prepared by the nitration of the dianhydride of sorbitol by concentrated nitric and sulphuric acids (Krantz et al, 1939a; Forman et al, 1941).

3.1.3. Ethylene Glycol Dinitrate

Ethylene glycol dinitrate (1,2-ethanediol dinitrate, nitroglycol) (EGDN) is a colourless oil which is prepared by the nitration of ethylene glycol with concentrated nitric and sulphuric acids. It was first synthesized by Henry in 1870 (Rinkenbach, 1927).

The structures of these organic nitrates are presented in Figure 3.1.

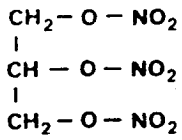
3.2. PHYSICAL PROPERTIES

Urbanski (1965) and Boschan (1955) have discussed in detail the physical properties of the organic nitrates. The lower nitrate esters are colourless sweet-smelling liquids. Because of the presence of a semi-polar bond in the nitro group, liquid organic nitrates have higher vapour pressures than their corresponding alcohols.

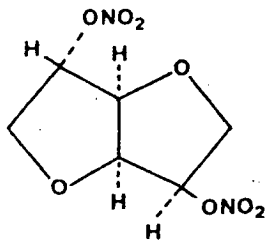
The organic nitrates have low water solubility. As the molecular weight of completely nitrated alcohols increases, water solubility decreases. They are very soluble in alcohol and other common organic solvents.

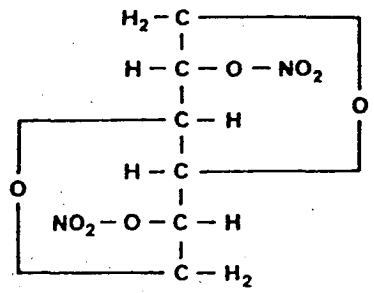
Some physical constants of interest are presented in Table 3.1.

NITROGLYCERIN



ISOSORBIDE DINITRATE





ETHYLENE GLYCOL DINITRATE

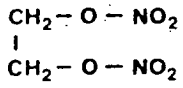


Figure 3.1 Structures of the organic nitrates of interest.

Table 3.1. Physical Constants of Organic Nitrates[a]

COMPOUND	Physical State at Room Temp.	Molecular Weight	Specific Gravity (gm/cc^3) at 20°C)	Water Solubility (g/litre at 20°C)	Melting Point (°C)	Vapour[b] Pressure (mm Hg at 20°C)
ETHYLENE GLYCOL DINITRATE	OIL	152.03	1.49	6.8	-22	0.038
NITROGLYCERIN	OIL	227.09	1.60	1.2-1.8	2.2 (labile) 13.2 (stable)	0.00026
ISOSORBIDE DINITRATE	SOLID	236.14	-	0.68	68-71	-

(a) Adapted from "Organic Nitrates," editor P. Needleman, Springer-Verlag (1975) p. 17.

(b) Urbanski (1965).

3.3. CHEMICAL STABILITY

3.3.1. Nitroglycerin

Nitroglycerin is hydrolyzed rapidly in alkaline solution. The hydrolytic reaction of nitroglycerin performed in an aqueous or alcoholic solution of sodium or potassium hydroxide results in the formation of organic acids, nitrates, nitrites, aldehyde resins and ammonia (Urbanski, 1965). This alkaline hydrolysis reaction has been used by Fung et al (1973) to develop a kinetic assay of nitroglycerin in aqueous solutions (see Section 3.4.). The rate of alkaline hydrolysis is much greater than acid hydrolysis (Crew and DiCarlo, 1968). Nitric oxides are formed upon the oxidation of nitroglycerin by nitric acid.

Boschan et al (1955) have reviewed the literature reports on the influence of temperature on the decomposition of the organic nitrates. Their review concentrated on ethyl nitrate.

Waring and Krastins (1970) found that above 140°C the thermal decomposition of pure nitroglycerin is approximately first order in nature. Autocatalytic effects influence the rate constant as the reaction proceeds at this temperature. The rate of thermal decomposition of nitroglycerin is doubled for every 5°C increase in temperature from 95°C to 125°C (Urbanski, 1965).

The stability of nitroglycerin in various solvents has been studied by Suphajettra et al (1978). Absolute alcohol, propylene glycol, glycerol, povidone and polyethylene glycol 400 were the solvents studied. At the elevated temperatures of accelerated stability studies, considerable loss of nitroglycerin was demonstrated only in polyethylene glycol 400.

Nitroglycerin appears to be unaffected by diffuse daylight, however concentrated ultraviolet rays in the 3200-4000nm range can initiate decomposition. The effect of heating previously irradiated nitroglycerin at 100°C quickly raises the reaction rate (Urbanski, 1965).

3.3.2. Isosorbide Dinitrate

Krantz et al (1962) found that isosorbide dinitrate could not be hydrolyzed after treatment with 0.2N sodium hydroxide at 37°C for 5 minutes. However, it has been reported that in alkaline and acidic media under vigorous hydrolytic conditions degradation of isosorbide dinitrate occurs (Silvieri and DeAngelis, 1975). Heating isosorbide dinitrate in 1N hydrochloric acid at 100°C for one hour resulted in 25% decomposition of the drug, and heating the drug in 1N sodium hydroxide at 100°C for one hour resulted in 45% decomposition.

3.3.3. Ethylene Glycol Dinitrate

Like nitroglycerin, ethylene glycol dinitrate is readily hydrolyzed in ethanolic or aqueous solutions of potassium or sodium hydroxide to give nitrite ion and glycolate. It is more stable in concentrated acids than nitroglycerin. Ethylene glycol dinitrate is hydrolysed to some extent by hot water (at 60°C, 0.008% over 5 days), the degree of hydrolysis being more than that of nitroglycerin (Urbanski, 1965).

3.4. METHODS OF ANALYSIS OF THE ORGANIC NITRATES

The earliest recorded method used to identify nitroglycerin involved ether extraction of the drug from the stomach of a cadaver, evaporation of the ether on an anvil and striking the extract with a hammer to see if it exploded (DiCarlo, 1975). Methods of analysis of nitroglycerin have generally improved since the above technique was used.

3.4.1. Colorimetry

Bogaert (1975) and DiCarlo (1975) have reviewed the colorimetric methods of analysis of nitroglycerin. Other organic nitrates to be analyzed in this way are ethylene glycol dinitrate and 1,2-propylene glycol dinitrate (Litchfield, 1968), 1-chloro-2,3-propanediol dinitrate and 1,2,3-pentanetriol trinitrate (Bell et al, 1963),

isosorbide dinitrate (Reed et al, 1971) and pentaerythritol tetranitrate (Das Gupta, 1978; Hankonyi and Karas-Gasparec, 1969).

After acid or alkaline hydrolysis of the parent compound to form nitrate or nitrite ion, the colorimetric reagents used include N-1-naphthylethylenediamine (Litchfield, 1968; Bell et al, 1963), m-xylenol (Reed et al, 1971), phenoldisulphonic acid (Das Gupta, 1978) and p-nitroaniline and azulene (Hankonyi and Karas-Gasparec, 1969).

3.4.2. Thin-Layer Chromatography

Thin-layer chromatographic methods (TLC) used to assay nitroglycerin have been reviewed by Bogaert (1975) and DiCarlo (1975). Investigators have used TLC for the separation of explosive mixtures of mannitol hexanitrate, pentaerythritol tetranitrate, diethylene glycol dinitrate, ethylene glycol dinitrate, nitroglycerin and sorbitol hexanitrate (Parihar et al, 1967), and to study the metabolism of pentaerythritol trinitrate in man (DiCarlo et al, 1977; Davidson et al, 1971), pentaerythritol tetranitrate in rats (Crew et al, 1975) and isosorbide dinitrate in dogs (Sisenwine and Ruelius, 1971). Quantitation of an organic nitrate and its metabolites has been facilitated by the use of radio-labeled parent compounds or by staining followed by densitometry. While

these methods are highly specific they lack sufficient speed and sensitivity to be of use in routine analyses of either pharmaceutical preparations or biological fluids.

3.4.3. Polarography

It has been known since 1933 that nitroglycerin is reducible at the dropping mercury electrode (Whitnack et al, 1955) and the mechanism of this reduction was studied by Kaufman et al (1952) and Radin and De Vries (1952). The behaviour of nitroglycerin, ethylene glycol dinitrate and pentaerythritol tetranitrate at the dropping mercury electrode was reported by Whitnack et al (1954) prior to the same workers developing a method for extraction and polarographic analysis of nitroglycerin in double-base explosive powder (Whitnack et al, 1955). Ayres and Leonard (1959) modified the assay of Whitnack et al (1955) to determine the pentaerythritol tetranitrate content when present in propellants with nitrolgycerin.

Following earlier work with the nitrate esters (Whitnack et al, 1954, 1955), Whitnack (1975) applied single sweep polarographic techniques in the detection of trace amounts of nitroglycerin and 1,2-propylene glycol dinitrate in ground and surface waters.

In the analysis of pharmaceutical preparations of nitrate esters, nitroglycerin has been assayed by both aqueous

(Flann, 1969) and non-aqueous (Woodson and Alber, 1969; Pugh, 1979) polarography, while Turner and Lenkiewicz (1976) showed that isosorbide dinitrate and the two isosorbide mononitrates are polarographically reducible and applied this method in the analysis of isosorbide dinitrate in several tablet formulations.

3.4.4. Infrared Spectroscopy

Pristera et al (1960) described infrared spectra of nitroglycerin, pentaerythritol tetranitrate, ethylene glycol dinitrate and diethylene glycol dinitrate and mentioned techniques for the quantitative analysis of mixtures of these compounds as occur in explosives. Whereas ultraviolet spectroscopy has become more popular than infrared techniques for the analysis of the above nitrate esters, isosorbide dinitrate exhibits an ultraviolet spectrum of only low sensitivity and specificity. For this reason Woo et al (1973) developed an infrared spectrometry method for the analysis of isosorbide dinitrate in dosage forms. This method provided a lower limit of detection of 12 mcg/ml solution and was specific and rapid.

3.4.5. Gas Chromatography

The early history of the development of gas chromatography for the analysis of nitroglycerin has been reviewed by

Bogaert (1975) and DiCarlo (1975). Alley and Dykes (1972) used a gas chromatograph equipped with a flame ionization detector to determine the nitroglycerin content of pharmaceutical preparations. Shortly after this, Rosseel and Bogaert (1973a) reported a method, using an electron-capture detector, capable of detecting nitroglycerin levels as low as 0.5 ng/ml plasma after buccal administration of nitroglycerin to man. This method was similar to one earlier reported by Sherber et al (1970) for the analysis of isosorbide dinitrate in rabbit blood except that Sherber et al (1970) used a flame ionization detector and consequently could only achieve a limit of detection for isosorbide dinitrate of 100 ng/ml plasma.

The method of Bogaert and Rosseel (1973a) has been the mainstay of analysis of nitroglycerin and isosorbide dinitrate in plasma with only modifications of the method (Blumenthal et al, 1977; Yap et al, 1978; Givant and Sulman, 1978; Armstrong et al, 1979; Wei and Reid, 1979; Assinder et al, 1977; Malbica et al, 1977; Laufen et al, 1978, Doyle et al, 1980) being subsequently reported in the literature. Other modified methods have been used in the analysis of 1,2- and 1,3-glyceryl dinitrates and 1- and 2-glyceryl mononitrates in blood (Neurath and Dunger, 1977), isosorbide 1- and 5-mononitrates in plasma (Richard et al, 1976; Chin et al, 1977; Rosseel and Bogaert, 1979) and nitroglycerin in intravenous fluids (Sturek et al, 1978).

Other organic nitrates to be separated or quantitated by gas chromatography are ethylene glycol dinitrate (Camera and Pravisani, 1964; Williams et al, 1966; Litchfield, 1968), diethylene glycol dinitrate, triethylene glycol dinitrate, 1,5-dinitropentanediol (Camera and Pravisani, 1964), ethylene glycol mononitrates and propylene glycol di- and mononitrates (Litchfield, 1968), pentaerythritol tetra-, tri-, di- and mononitrates (Davidson et al, 1971) and isomannide and isoidide dinitrates and mononitrates (Rosseel and Bogaert, 1972; Rosseel and Bogaert, 1973b).

3.4.6. Kinetic Assay

A kinetic method for the analysis of nitroglycerin in dosage forms was reported by Fung et al (1973) to be simpler than gas chromatographic and polarographic methods. Moreover the other simple method of analysis of nitroglycerin, the colorimetric method of Bell et al (1963), was reported by Morrison and Fung (1979) to be affected by inorganic nitrite ion.

Fung et al (1973) found that nitroglycerin degrades in alkaline methanolic solution in a stepwise mode with the formation of a chromophoric intermediate which has a wavelength of maximum absorbance of 328nm. The assay is unaffected by the presence of glycerol, nitrate and nitrite ions up to a concentration of 0.01M, and glyceryl

dinitrates and glyceryl mononitrates (Yap et al, 1975b). The kinetic assay was shown by Yap et al (1975a) to be also suitable for the determination of erythritol tetranitrate, pentaerythritol tetranitrate and mannitol hexanitrate in dosage forms. Waaler et al (1977) adapted this method to the automated analysis of nitroglycerin in tablets.

3.4.7. High-Pressure Liquid Chromatography

Normal phase high-pressure liquid chromatography (HPLC) has been used for the separation and identification of nitroglycerin and its dinitro metabolites in waste waters from an ammunition plant (Chandler et al, 1974) and for the separation and identification of nitroglycerin and its stabilizers and plasticizers in explosives (Doali and Juhasz, 1974). The findings of Camera and Pravisani (1964), showing that nitroglycerin appreciably decomposed while on the column of a gas chromatograph, made it apparent that the analysis of thermally labile nitrate esters would be more suited to high-pressure liquid chromatography. Moreover, because of the non-destructive nature of the analysis by ultraviolet detection, subsequent analysis of any separation product could be performed. These advantages are offset, however, by the great reduction in sensitivity of ultraviolet and fluorescence detectors when compared to electron-capture detection in conjunction with gas chromatography. The method produced by Chandler et al (1974) resulted in a lower level of

detection of 10 mcg/ml water for nitroglycerin and its two dinitro metabolites. Both the above HPLC methods required extraction of water samples (Chandler et al, 1974) or propellant mixtures (Doali & Juhasz, 1974) with methylene chloride.

Pentaerythritol, a metabolite of pentaerythritol tetranitrate, was analyzed as the tetra-p-methoxybenzoate derivative using an HPLC method that resulted in a lower limit of detection of pentaerythritol equivalent to about 2 mcg/ml plasma (Bighley et al, 1975). Solvent extraction of plasma by a mixture of heptane and chloroform was necessary in this method.

The analysis of nitroglycerin in dosage forms by reversed-phase HPLC was first performed by Crouthamel and Dorsch (1979). This method using a mobile phase of 40% methanol-water and C18 column was the first to resolve nitroglycerin, 1,2- and 1,3-dinitroglycerins and 1- and 2-mononitroglycerins. The lower limit of detection of nitroglycerin was 3 mcg/ml water.

Another HPLC assay procedure for nitroglycerin in tablet and intravenous dosage forms was reported by Baaske et al (1979). This assay used an alkyl phenyl column and an acetonitrile-water mobile phase for separation and a detection wavelength of 218nm. Isosorbide dinitrate was used as the internal standard for quantitation.

Fan et al (1978) described a method for the analysis of ethylene glycol dinitrate esters in water by means of a thermal energy analyzer and this method was applied by Spanggord and Keck (1980) to the analysis of nitroglycerin and its four metabolites in dogs' blood. Separation of all the degradation products from the parent compound was achieved on a Lichrosorb S-60 column using a gradient elution technique. Trace levels of organic nitrates in explosives were also identified using HPLC with a thermal energy analyzer (Lafleur and Morriseau, 1980).

CHAPTER 4

EXPERIMENTAL

4.1. MATERIALS

4.1.1. Chemicals

Acetic acid, glacial, analytical reagent, Ajax Chemicals, 62055

ammonium hydroxide, analytical reagent, Commonwealth Ammonia Corporation

benzene, analytical reagent, Ajax Chemicals

chlormethiazole edisylate, Astra Pharmaceuticals, DC 1750, lot 8976

diazepam, Roche Products

diethyl ether, analytical reagent, Ajax Chemicals, 64052

diphenylamine, British Drug Houses

ethyl acetate, analytical reagent, Ajax Chemicals, 82837

ethylene glycol dinitrate (5% w/v), I.C.I. Operations

hexane, spectroscopic grade, Ajax Chemicals, 83092

isosorbide dinitrate, 40% on lactose, Schweiz

isosorbide mononitrates, Ayerst Laboratories

methanol, analytical reagent, Ajax Chemicals, 83248 and chromatographic grade, Waters Associates, 24626M

nitroglycerin (5% v/v), Dept. Manufacturing Industry

octanol, laboratory quality, BDH Chemicals, 29409

phenol, analytical reagent, May and Baker, 45693

potassium nitrate, analytical reagent, By-Products and Chemicals, 50506

silica gel, GF, Sigma Chemical Company, lot 25C-0219

sodium hydroxide, Volucon N/1, May and Baker, lot A9

sulphuric acid, concentrated, analytical reagent, Ajax Chemicals, 303308

4.1.2. General

All glassware was washed in 'Pyroneg', rinsed with distilled water and then washed with methanol before drying in an oven at 70°C. The distilled water used throughout was produced by a Buchi all-glass still.

Plastic intravenous infusion bags (Viaflex, code AHB1323) and plastic giving sets (Buretrol, code 2C0133 and AHC0132) were received from Travenol Industries, Melbourne.

High density polyethylene tubing ('Intramedic PE50', Clay Adams, New Jersey) was attached to the outlet of a 50ml interchangeable glass syringe (Ultra-Asept, West Germany) which was driven by an infusion pump (Type 871104, B. Braun, Melsungen).

Because the concentration of nitroglycerin in the 5ml bottles supplied varied from bottle to bottle, the nitroglycerin content of each bottle was standardized using the method of Dean and Baun (1975).

Isosorbide dinitrate (40%) on lactose was purified by ether extraction of the powder and recrystallization from an ethanol/water mixture.

Ethylene glycol dinitrate was used as received (500mls of 5% w/v EGDN in ethanol).

4.2. STORAGE STUDIES AND SIMULATED INFUSIONS

4.2.1. Plastic Infusion Bags

Glass syringes were used to transfer known volumes of ethanolic stock solutions of nitroglycerin, isosorbide dinitrate or ethylene glycol dinitrate through the administration port of infusion bags containing 500mls of either 5% dextrose in water, normal saline or water. The solutions were mixed thoroughly by drawing solution into the barrel of a glass syringe and then squirting the drawn solution back into the contents of the bag. This process was repeated several times. An aliquot of solution was removed immediately after mixing and this sample was regarded as the zero-time sample. All air was removed from the contents of each bag. Bags were then supported in an upright position so that all exterior surfaces were exposed to the air. Thereafter samples were taken from the aqueous solutions in the bags, after mixing in the above-mentioned way, at known time intervals.

When the effect of the volume of drug solution on the loss of nitroglycerin from solution stored in the plastic bags was investigated, various volumes were initially removed from the bags and known volumes of stock drug solutions were added to the remaining solution so that all volumes studied were equivalent in drug concentration. Studies of the loss of the test drugs from their aqueous solutions stored in plastic infusion bags were performed over a range of temperatures, however "volume effect" studies were only performed at ambient temperature (20°-24°C).

4.2.2. Isolated Burettes of Plastic Giving Sets

Storage studies involving the loss of nitroglycerin, isosorbide dinitrate or ethylene glycol dinitrate from aqueous solutions stored in the isolated burettes of giving sets were performed in a similar manner to studies using the infusion bags (Section 4.2.1.) except that accelerated stability testing was not undertaken. The burettes were separated from the giving sets and their outlets were closed with metal screw clamps. The burette holds a maximum volume of 150mls of solution and this volume was normally used except when studying a possible "volume effect" on the loss of nitroglycerin from solution. In this case, different volumes of the same drug solution were added directly to the burette. Studies were performed at ambient temperature (20°-24°C).

4.2.3. Plastic Infusion Tubing

The disappearance of nitroglycerin from aqueous solutions stored in the plastic infusion tubing of the giving sets was studied using 2-cm lengths of polyvinyl chloride tubing filled with aqueous nitroglycerin solution. Both ends of each piece of tubing were closed by means of metal screw clamps. A series of pairs of pieces of tubing was set up so that at each sampling time the drug solution from a new pair was sampled. This method was necessary because the kinetic assay of nitroglycerin (see Section 4.6.1.) used a large proportion of the test solutions stored in the small pieces of tubing. These studies were performed at ambient temperature (20°-24°C).

All storage studies were performed at least in duplicate. As well, an aliquot of the test solution, sampled at zero time, was stored in glass bottles with metal screw caps and analyzed for drug content at intervals throughout the duration of each storage experiment.

4.2.4. Simulated Infusions

(a) Plastic Giving Sets

The simulated infusion of aqueous solutions of nitroglycerin and isosorbide dinitrate to patients was

performed using glass infusion bottles or plastic infusion bags connected to either whole giving sets or the isolated tubing of giving sets.

For all studies in which burettes were employed, the volume of drug solution in the burette was maintained at 100ml. Drug solution was allowed to initially flow from the glass bottle or plastic bag through the tubing of the intact giving set or the isolated tubing until 5 drops of effluent were collected. By means of a measuring cylinder and a stop watch, a desired flow rate was then set. Infusion rates were controlled manually and were checked regularly to maintain a constant flow rate.

(b) High Density Polyethylene Tubing Connected to a Glass Syringe

Nitroglycerin, isosorbide dinitrate, diazepam and chlormethiazole solutions were pumped from a 50ml glass syringe via a high density polyethylene cannula (80 x 0.15cm). The glass syringe was driven by a Braun infusion pump to give a predetermined constant flow rate. Effluent was collected from the end of the tubing for analysis.

All simulated infusions were performed in duplicate at ambient temperature (20°-24°C).

4.3. KINETIC STUDIES WITH PLASTIC SHEETS AND TUBING

4.3.1. Sorption Studies

Pieces of polyvinyl chloride infusion bag (1cm x 1cm), cellulose propionate burette (1cm x 1cm) and finely cut-up high density polyethylene tubing (2cm) were carefully weighed before being immersed in 5ml (polyvinyl chloride and cellulose propionate samples) or 0.4ml (high density polyethylene tubing) of aqueous drug solution. Solutions were stored in glass-stoppered 10 or 1ml glass tubes which were shaken vigorously at regular intervals throughout the course of the experiments. Aliquots of solution were removed from the containers at the beginning of an experiment and again at known intervals thereafter until equilibrium was reached. Solutions were stored in duplicate at 4°+/-1°C, room temperature (20°-24°C), 37°+/-1°C, 45°+/-1°C and 60°+/-1°C.

4.3.2. Permeation Studies

Studies were designed to determine whether the loss of any of the three organic nitrates from their aqueous solutions stored in contact with the polyvinyl chloride infusion bags was due to permeation of the drug through the plastic bag into the external environment and these studies involved using the apparatus depicted in Figure 4.1. The diffusion

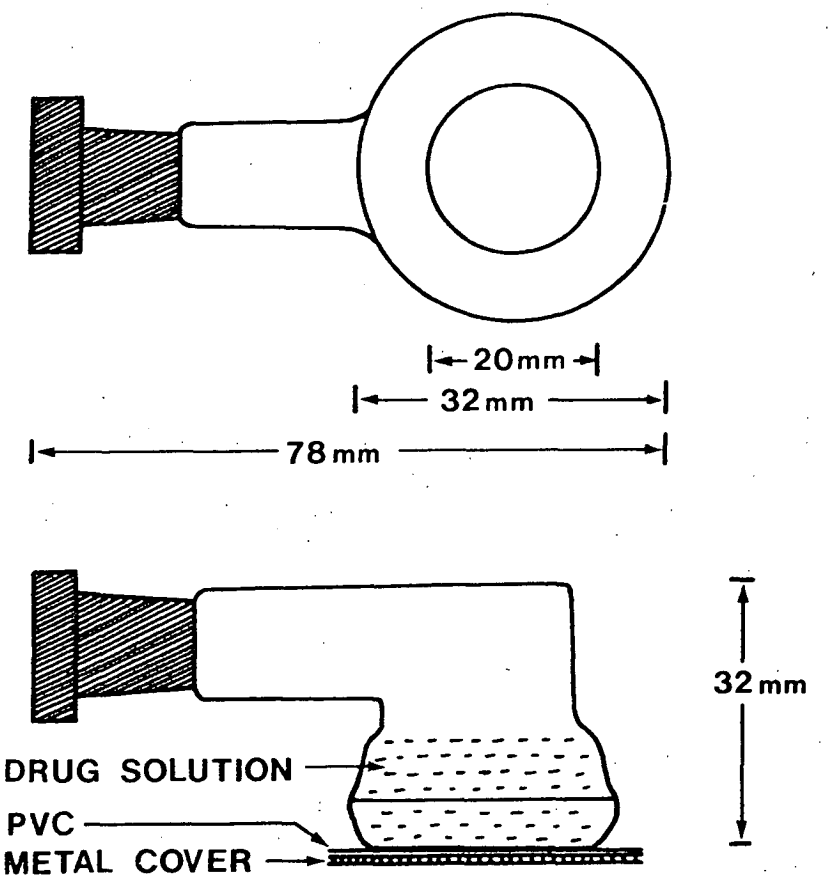


Figure 4.1 Diagrammatic representation of the apparatus used in permeation studies. The PVC sheet is held sandwiched between the drug solution and the metal cover by a metal clamp.

cell had pieces of polyvinyl chloride (3.5cm x 3.5cm x 0.039cm), cut from infusion bags, held firmly in place across the well of the diffusion cell by means of either annular or solid square sheets of stainless steel and a metal clamp. The solid metal sheets were intended to prevent drug molecules escaping to the outside air, while the annular sheets were intended to not inhibit the passage of drug molecules to the outside air.

Aliquots of drug solutions were removed for analysis from the neck of the apparatus. Solutions of nitroglycerin were stored in the diffusion cells at room temperature and 45°+/-1°C while solutions of isosorbide dinitrate and ethylene glycol dinitrate were stored at only 45°+/-1°C. All tests were performed in duplicate.

4.4. PARTITION COEFFICIENTS

Two millilitres of hexane or octanol, previously saturated with water, were pipetted into a 10ml glass tube. Five millilitres of a solution of known concentration of nitroglycerin, isosorbide dinitrate or ethylene glycol dinitrate in water (saturated with hexane or octanol prior to adding the test drugs) were added and the tubes stoppered. Containers were stored at a range of temperatures and were shaken vigorously at regular intervals. Aliquots were removed from the aqueous phase periodically for analysis until equilibrium was reached.

Octanol-water and hexane-water partition coefficients were calculated as the ratio of equilibrium concentrations of the three test drugs in the organic phase and in the aqueous phase. Plastic-water partition coefficients were calculated using the results of the equilibrium sorption studies (see Section 4.3.). The concentrations of drugs in the plastic and in the aqueous solutions were calculated as weight of drug per weight of plastic or water.

4.5. DRUG METABOLITES

Because authentic samples of the glyceryl dinitrates and ethylene glycol mononitrate were not commercially available, they were obtained by means of incubating nitroglycerin or ethylene glycol dinitrate with fresh human blood (Lee, 1973; Clark and Litchfield, 1967).

About 500mcg of nitroglycerin or ethylene glycol dinitrate was added to 10ml of fresh human blood and the mixture was incubated at 37°C for one hour. Diethyl ether (2ml) was used to extract 1ml aliquots of the blood and 20 microlitres (ul) of this extract was injected directly into the HPLC system. These metabolites were needed so that their retention times during HPLC could be obtained in case possible hydrolysis products of the parent drug in solution produced interfering peaks in the chromatograms.

4.6. ANALYTICAL METHODS

4.6.1. Kinetic Assay

For initial work involving the loss of nitroglycerin from aqueous solutions stored in plastic infusion bags, burettes of giving sets and plastic tubing, a modified version of the kinetic assay of Fung et al (1973) (see Section 3.4.6.) was used. One millilitre of test aqueous solution was mixed with 2ml of 0.6N methanolic sodium hydroxide. In the present work, a blank solution consisting of 1ml of sample and 2ml of water was used for reference.

The composition of methanolic sodium hydroxide solution was as follows:

1N NaOH.....60ml
Methanol.....28ml
Water.....12ml

Analyses were performed on a Cecil Instruments Ultraviolet Spectrophotometer Model CE202.

4.6.2. High-Pressure Liquid Chromatography

A modified version of two previously reported methods for the analysis of nitroglycerin in aqueous solutions

(Crouthamel and Dorsch, 1979; Baaske et al, 1979) was used for the analysis of nitroglycerin, isosorbide dinitrate and ethylene glycol dinitrate.

Aliquots (20ul) of the sample solutions were injected into a Waters Associates high-performance liquid chromatograph equipped with a Micro-Bondapak C18 column (Waters). A mobile phase of water and methanol (60:40) was pumped at a flow rate of 2 ml/min. A Waters Model 450 Variable wavelength detector set at 218nm was used for quantitation.

Standard curves were constructed with and without the use of a standard reference compound. When a reference compound was used it was one of the two organic nitrates not under examination at the time. In this case 200ul of the reference solution (500 mcg/ml) was added to 1ml of test solution and the mixture was vortexed for 10 seconds before a 20ul aliquot was removed and injected into the chromatographic system. The ratio of peak heights of the test and reference compounds was then calculated and the concentration of test drug was read from a standard curve.

When a reference compound was not used, the peak height (in mm) of the test drug was measured and its concentration was read from a standard curve. When this method was used to analyze solutions in contact with plastic for extended periods, the method was periodically calibrated by use of stock solutions of three known concentrations of the test

drugs.

4.6.3. Spectrophotometry

Aqueous solutions of chlormethiazole and diazepam were analyzed by spectrophotometric methods. Chlormethiazole was diluted 1 to 200 with water and the optical density measured at a wavelength of 257nm. Diazepam samples were diluted 1 to 1 with 0.2N H_2SO_4 and the optical density measured at a wavelength of 275nm.

4.6.4. Thin-Layer Chromatography

To authenticate the products of the incubation of nitroglycerin or ethylene glycol dinitrate in fresh human blood (Section 4.5.), diethyl ether extracts of the incubation mixture were spotted onto silica gel plates and developed using the method of Needleman and Hunter (1965).

CHAPTER 5

RESULTS

5.1. ANALYTICAL METHODS

5.1.1. Kinetic Assay

The composition of reactant solution used for the kinetic assay (see Section 4.6.1.) resulted in a maximum absorbance at about 5 minutes for nitroglycerin in water or normal saline solution, and at about 6 minutes for nitroglycerin in 5% dextrose in water solution.

Standard curves constructed for nitroglycerin in water, normal saline and 5% dextrose in water solutions are shown in Figure 5.1. The level of detection was less than 3 mcg/ml solution and linearity was observed down to a concentration of about 10 mcg/ml solution. Below this concentration the calibration plot curved to pass through the origin.

5.1.2. High-Pressure Liquid Chromatography

The mobile phase and flow rate used in this work (see Section 4.6.2.) gave retention times for ethylene glycol dinitrate, isosorbide dinitrate and nitroglycerin of 5.8, 7.0 and 9.5 minutes respectively (Figure 5.2).

Standard curves constructed both with and without a reference compound were linear in the range 0-400 mcg/ml solution and passed through the origin (Figures 5.3, 5.4, 5.5). The limit of detection of nitroglycerin, isosorbide dinitrate and ethylene glycol dinitrate was less than 1 mcg/ml solution.

The results of the reproducibility studies for the quantitation of nitroglycerin, isosorbide dinitrate and ethylene glycol dinitrate with and without a reference compound are presented in Table 5.1. These show that the assays are both accurate and precise. Therefore an internal standard was not routinely used.

The organic nitrates can be assayed by a number of techniques (see Section 3.4.) but apart from gas chromatography and high-pressure liquid chromatography there is no method by which nitroglycerin, isosorbide dinitrate and ethylene glycol dinitrate as well as their degradation products can be measured using exactly the same analytical conditions. Gas chromatography, however, would involve time-consuming extraction from aqueous solutions into an organic solvent. For this reason, a high-pressure liquid chromatography assay was chosen.

The hydrolysis products of the three parent drugs had different retention times to each of the respective parent

drugs. However, if isosorbide dinitrate was used as an internal standard for the quantitation of nitroglycerin, it had a similar retention time as that of 1,3-glyceryl dinitrate, a potential hydrolysis product of nitroglycerin. This problem was not encountered in practice because an internal standard was not routinely used.

The three parent organic nitrates displayed greater absorbance at a wavelength of 218nm than at 200nm which is very close to the cut-off point for the methanol used in the mobile phase. Samples from bags containing only 5% dextrose in water, normal saline or water produced no extraneous peaks in the chromatograms.

5.1.3. Comparison of Kinetic and HPLC Methods

To determine the precision of the HPLC method of analysis of nitroglycerin compared to the kinetic assay, standard solutions of nitroglycerin containing 200 mcg/ml and 50 mcg/ml were assayed 10 times by both methods. The results are given in Table 5.2. The precision of the two methods is similar.

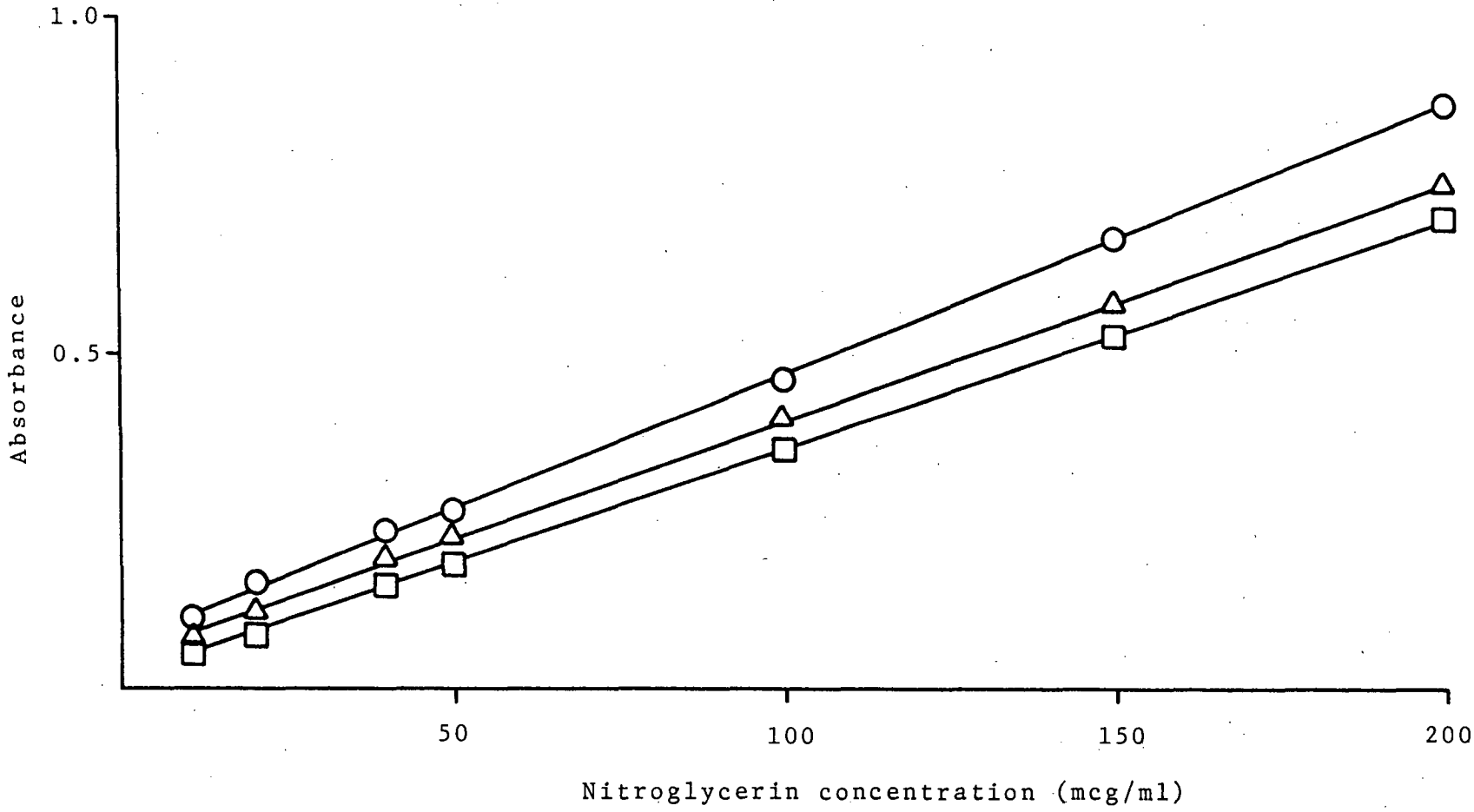


Figure 5.1 Standard curves for the kinetic assay of nitroglycerin in 5% dextrose in water (○), normal saline (△) and water (□).

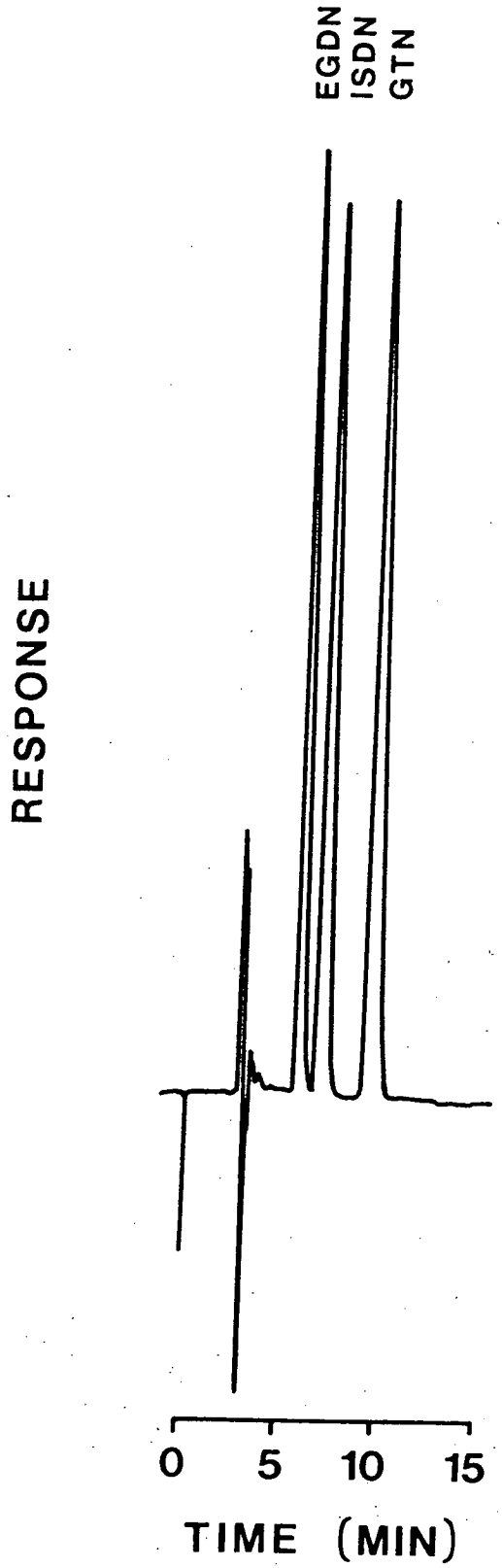


Figure 5.2 High-pressure liquid chromatogram of a mixture of nitroglycerin (GTN), isosorbide dinitrate (ISDN) and ethylene glycol dinitrate (EGDN) at final concentrations of 80 mcg/ml water.
(Chromatographic conditions are given in the text).

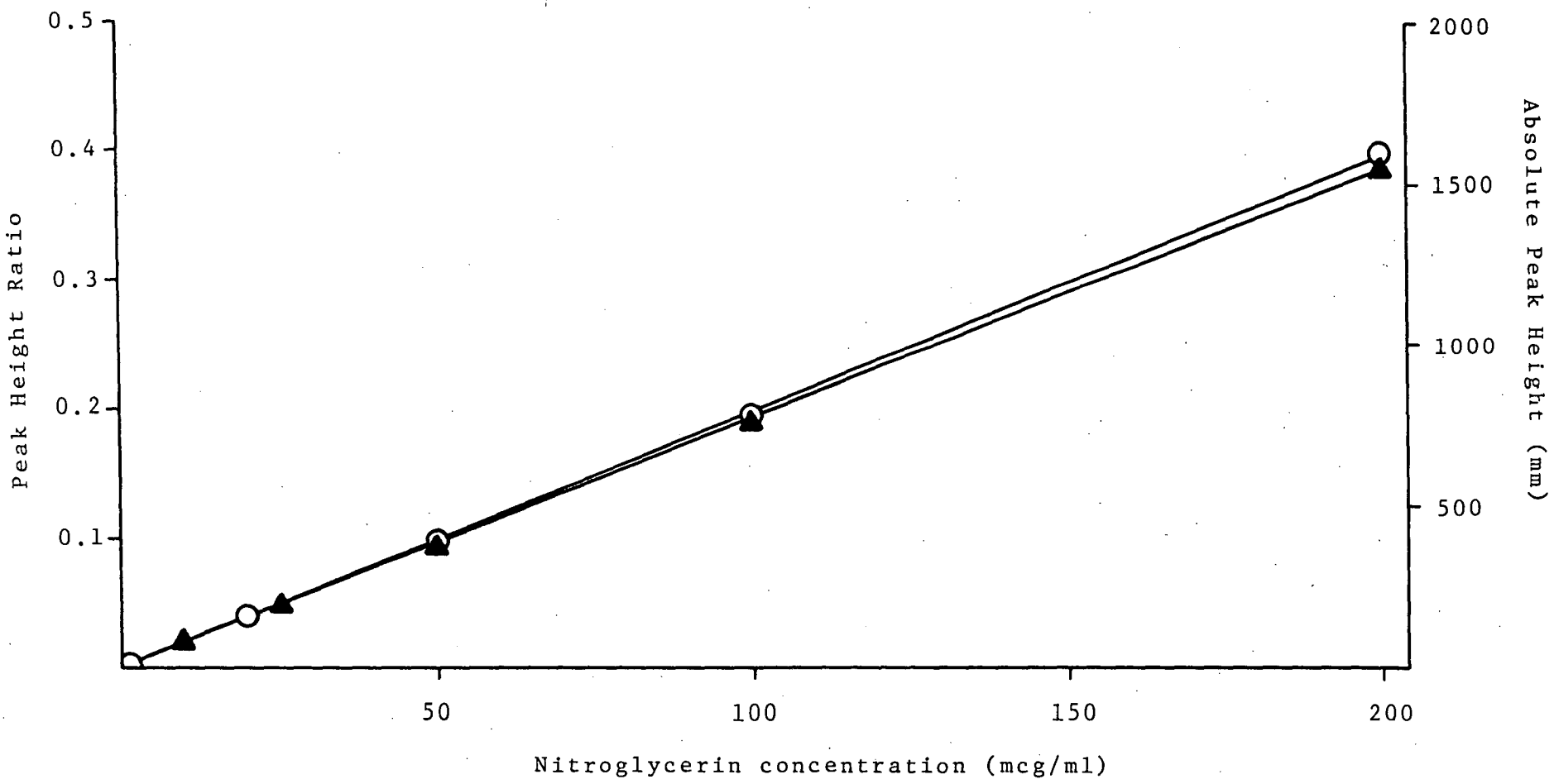


Figure 5.3 Standard curve for the HPLC assay of nitroglycerin using peak height ratio (peak height of drug/peak height of internal standard, isosorbide dinitrate) (▲) and absolute peak height (○).

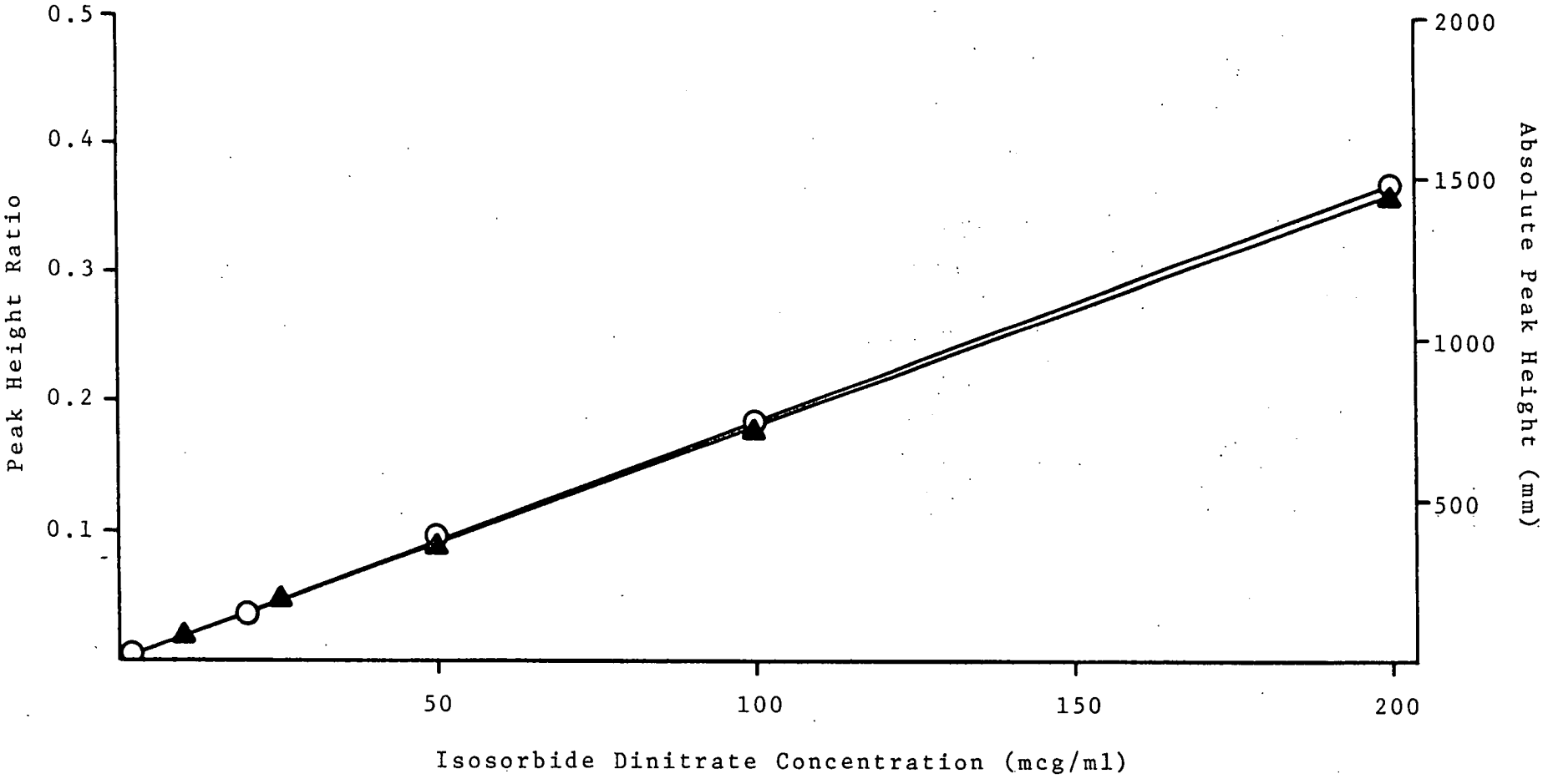


Figure 5.4 Standard curve for the HPLC assay of isosorbide dinitrate using peak height ratio (peak height of drug/peak height of internal standard, ethylene glycol dinitrate) (▲) and absolute peak height (O).

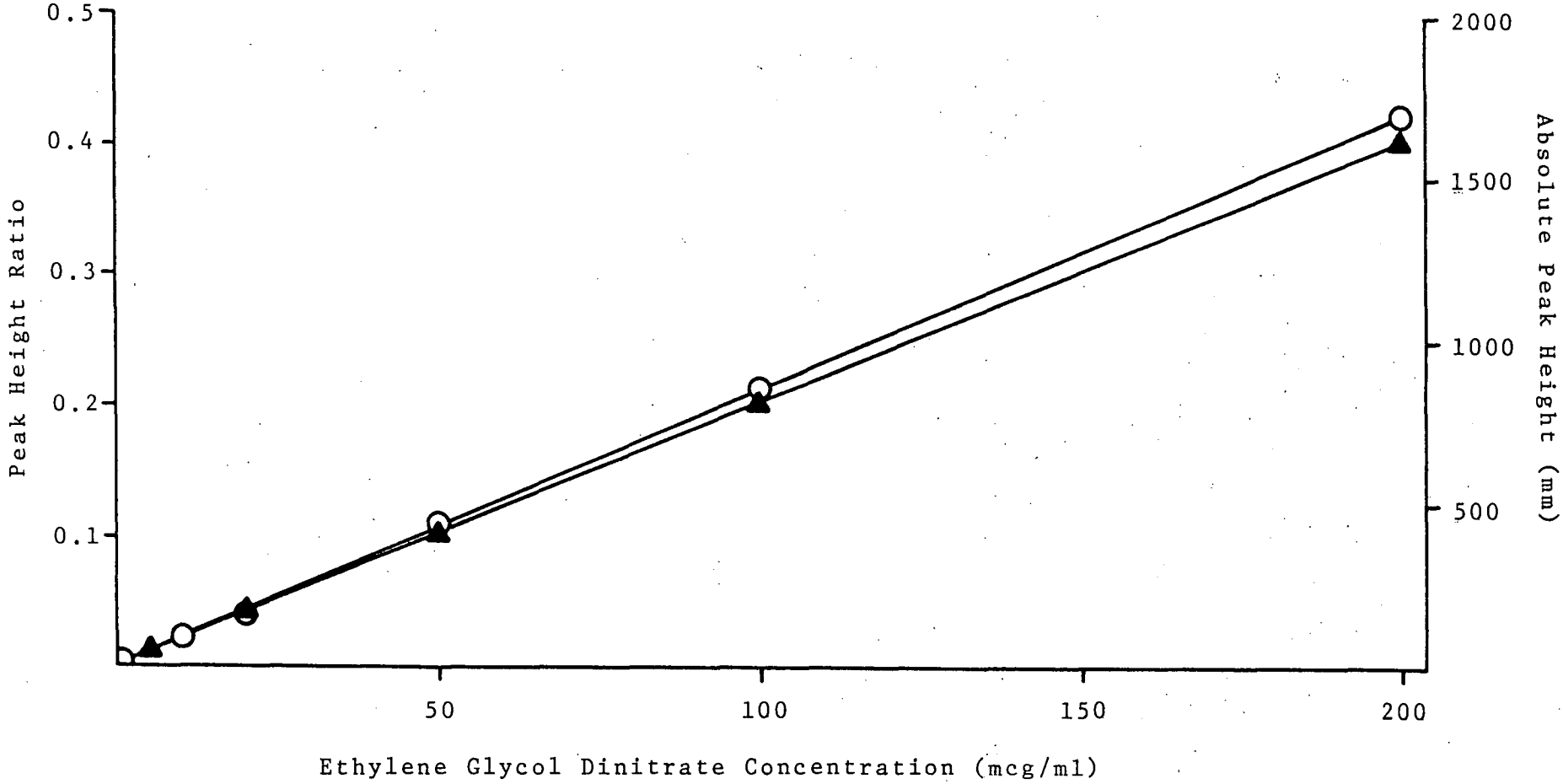


Figure 5.5 Standard curve for the HPLC assay of ethylene glycol dinitrate using peak height ratio (peak height of drug/peak height of internal standard, isosorbide dinitrate) (▲) and absolute peak height (O).

Table 5.1. Reproducibility of the HPLC assay of nitroglycerin (GTN), isosorbide dinitrate (ISDN) and ethylene glycol dinitrate (EGDN) based on peak height ratio (PR) of organic nitrate to internal standard and on absolute peak heights (PH).

Parameter	GTN		ISDN		EDGN	
	PR	PH	PR	PH	PR	PH
Mean concentration (mcg/ml)	200.1	199.9	199.5	200.1	200.1	199.6
Standard deviation	1.73	1.95	2.27	2.09	1.79	1.82
CV, %	0.86	0.97	1.13	1.04	0.89	0.91
Mean concentration (mcg/ml)	49.7	50.2	49.9	49.5	49.7	50.0
Standard deviation	0.97	0.83	0.91	0.93	0.81	0.76
CV, %	1.83	1.66	1.82	1.87	1.63	1.53

Table 5.2. Comparison of the Reproducibility of the Kinetic and HPLC Assays for Nitroglycerin

Parameter	Kinetic Assay n=10	HPLC Assay n=10
Mean concentration (mcg/ml)	200.0	199.9
Standard deviation	1.66	1.95
CV, %	0.83	0.97
Mean concentration (mcg/ml)	50.1	50.1
Standard deviation	0.76	0.83
CV, %	1.52	1.66

5.2. STORAGE STUDIES AND SIMULATED INFUSIONS

5.2.1. Initial Infusion

At the Royal Hobart Hospital infusions of nitroglycerin are usually commenced at a flow rate of 0.04 ml/min (16 mcg/min). The rate is then doubled every 5 minutes until a maximum flow rate of 0.64 ml/min (256 mcg/min) is reached. The infusion is maintained at this maximum flow rate thereafter. The results of this simulated study using plastic infusion bags and giving sets are shown in Figure 5.6. There was an initial rapid decrease in the concentration of nitroglycerin in the effluent followed at longer times by a small increase in the concentration of nitroglycerin up to a relatively stable plateau level which was maintained throughout the 7hr simulated infusion.

5.2.2. Vehicle

The time course of loss of nitroglycerin, isosorbide dinitrate and ethylene glycol dinitrate from solutions stored in plastic infusion bags, isolated burettes of giving sets or plastic tubing of giving sets at room temperature is shown in Figures 5.7 to 5.11. The loss was independent of the nature of the vehicle, the profiles for any one drug being similar for drug solutions in 5% dextrose in water, normal saline and water.

Drug solutions stored in glass under the same conditions lost no potency.

5.2.3. Concentration

The percentage of nitroglycerin, isosorbide dinitrate and ethylene glycol dinitrate remaining in aqueous solutions stored in plastic infusion bags or isolated burettes of giving sets was independent of the concentration of drug in the range 100 mcg/ml to 1000 mcg/ml. Representative profiles of loss of these drugs are illustrated in Figures 5.7 to 5.10.

No loss of the test drugs occurred when drug solutions were stored in glass.

5.2.4. Volume

Figure 5.12 shows the effect of solution volume on the percentage of nitroglycerin remaining in solutions stored in infusion bags and burettes for various times. For the infusion bags, the percentage of nitroglycerin remaining in solution decreased more rapidly for the smaller volumes (Figure 5.12a). The percentage of nitroglycerin remaining in aqueous solutions stored in the burettes for various times appears to be independent of the volume of solution in the burette (Figure 5.12b).

5.2.5. Temperature

The effect of temperature on the loss of nitroglycerin, isosorbide dinitrate and ethylene glycol dinitrate from their aqueous solutions stored in plastic infusion bags is seen in Figures 5.13 to 5.15. The rate of loss was slowest at 4°C and this rate increased markedly with increasing temperature.

Nitroglycerin and ethylene glycol dinitrate were completely lost from solution at the higher temperatures. However, isosorbide dinitrate was not completely lost from solution even at 60°C.

There was no loss of any of the test drugs when stored in glass at elevated temperatures.

5.2.6. Light

There were no differences in the rate or extent of loss of nitroglycerin when 100 mcg/ml and 500 mcg/ml nitroglycerin solutions were stored in plastic infusion bags in the dark or in room light at ambient temperature.

5.2.7. Flow Rate

(a) Nitroglycerin

The flow rate dependent loss of nitroglycerin from aqueous solutions being infused from glass bottles through giving sets is illustrated in Figure 5.16. The percentage of the original nitroglycerin concentration which remained in the eluting solution was greater at faster flow rates. Nitroglycerin concentration decreased rapidly within the first hour at all the flow rates used, but with the faster flow rates, a subsequent increase occured before a plateau level was reached. For the fastest flow rate this level was about 70% of the original nitroglycerin concentration.

When solutions of nitroglycerin were infused from glass bottles through plastic infusion tubing, the concentration of nitroglycerin in the effluent diminished immediately after beginning the infusion and then gradually increased for longer times (Figure 5.17a). The cumulative amount of nitroglycerin sorbed by the infusion tubing at different flow rates is plotted as a function of time in Figure 5.17b.

Figure 5.18a illustrates the loss of nitroglycerin from solutions infused from various systems at a constant flow rate. In Figure 5.18b the cumulative amount of nitroglycerin sorbed by the components of the various

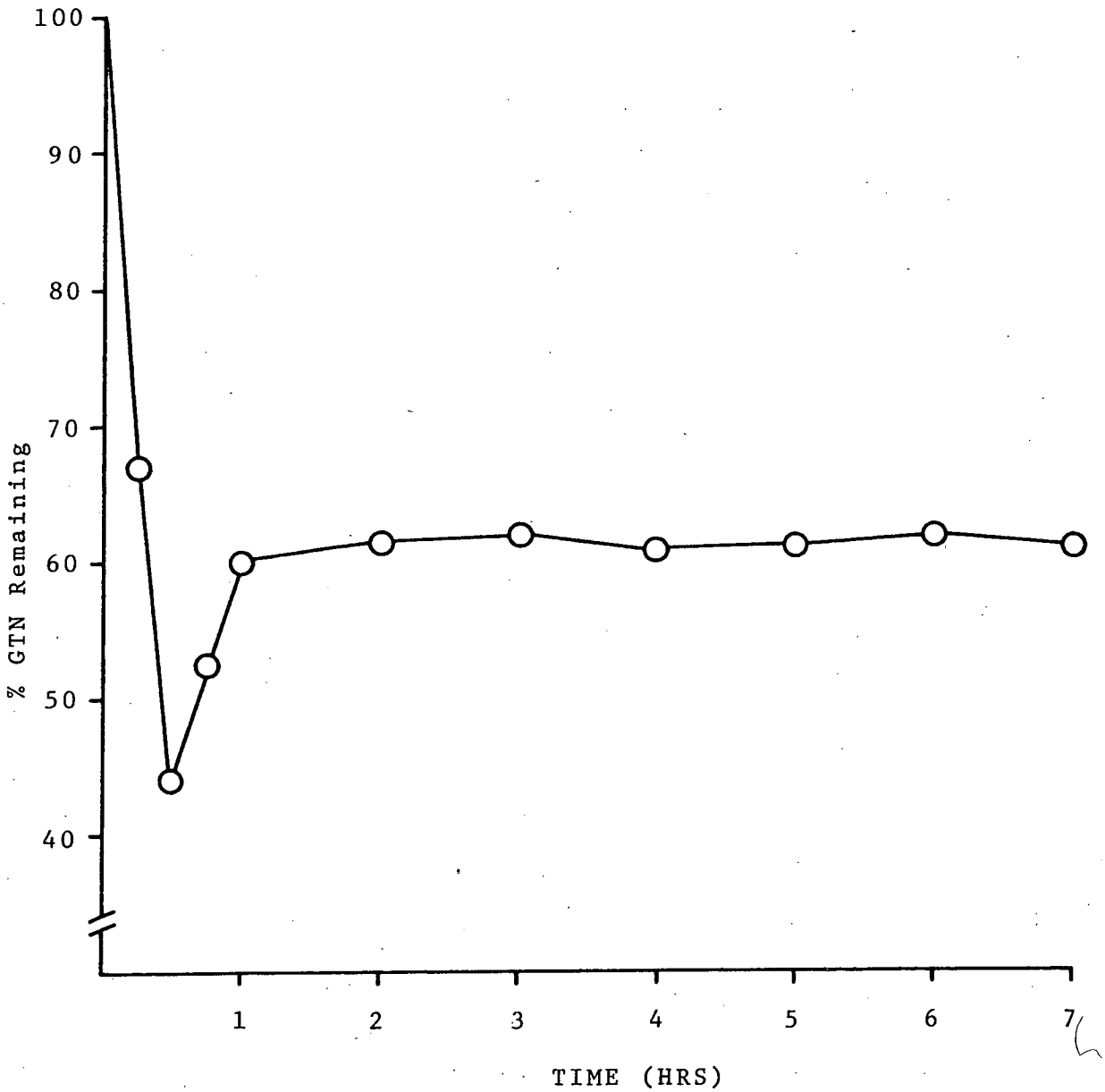


Figure 5.6 Initial simulated infusion of nitroglycerin (GTN) from plastic infusion bag through plastic giving set at a variable flow rate (see text for details).

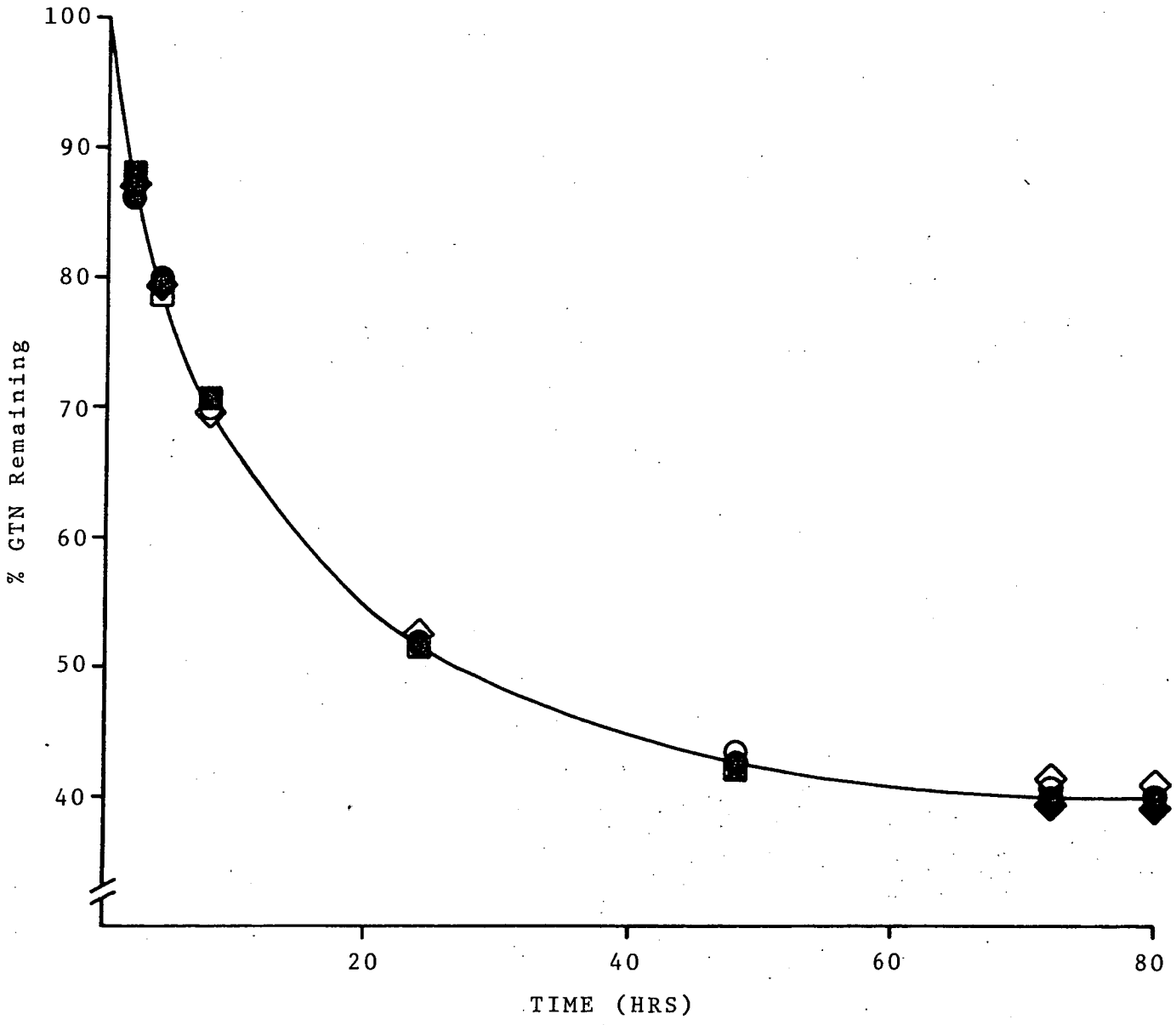


Figure 5.7 Percentage of nitroglycerin (GTN) remaining in solutions stored in plastic infusion bags for original nitroglycerin concentrations of 100 mcg/ml (open symbols) and 1000 mcg/ml (closed symbols). ○ ● water; □ ■ 5% dextrose in water; ◇ ◆ normal saline.

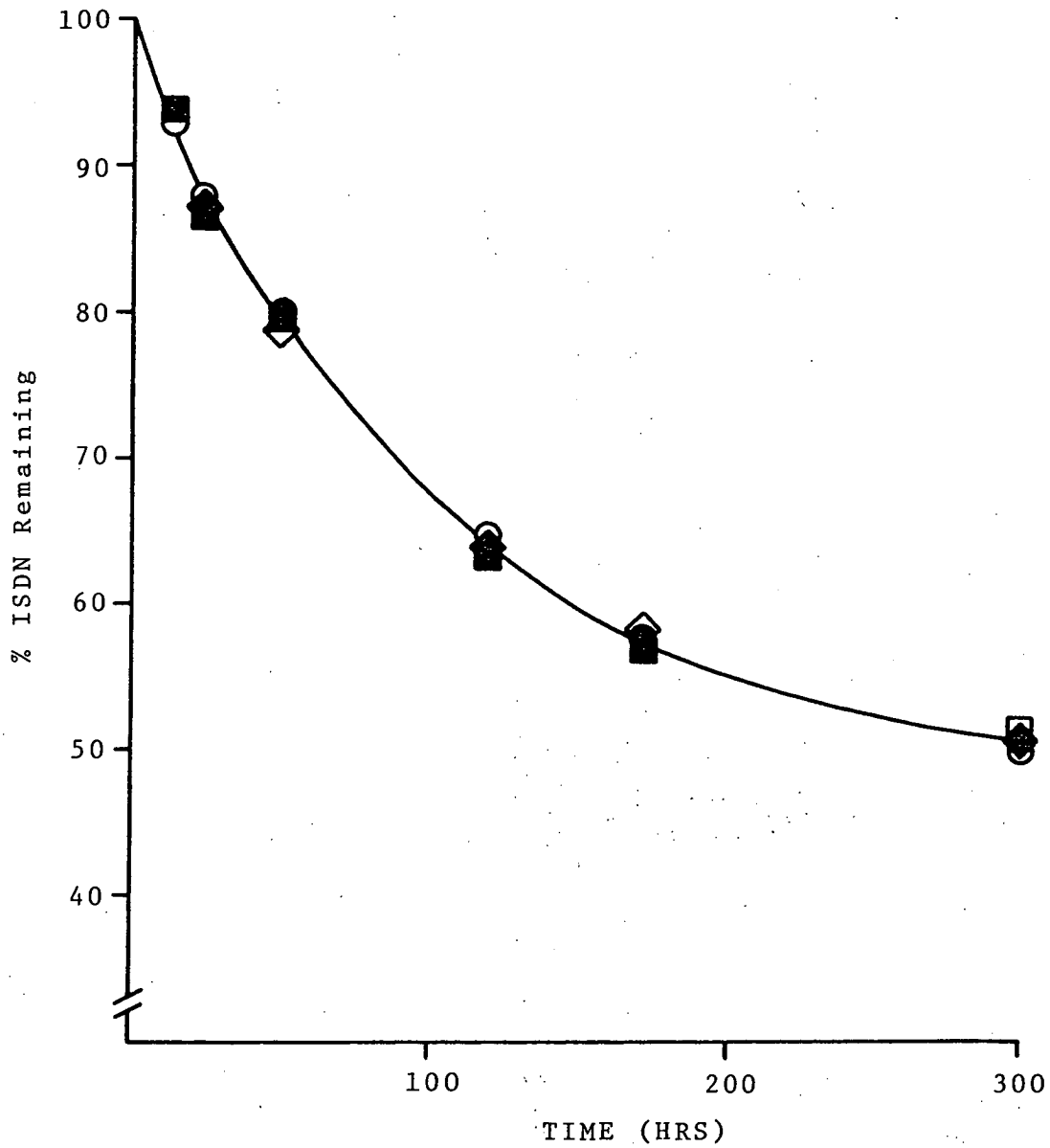


Figure 5.8 Percentage of isosorbide dinitrate (ISDN) remaining in solutions stored in plastic infusion bags for original isosorbide dinitrate concentrations of 100 mcg/ml (open symbols) and 1000 mcg/ml (closed symbols). ○ ● water; □ ■ 5% dextrose in water; ◇ ◆ normal saline.

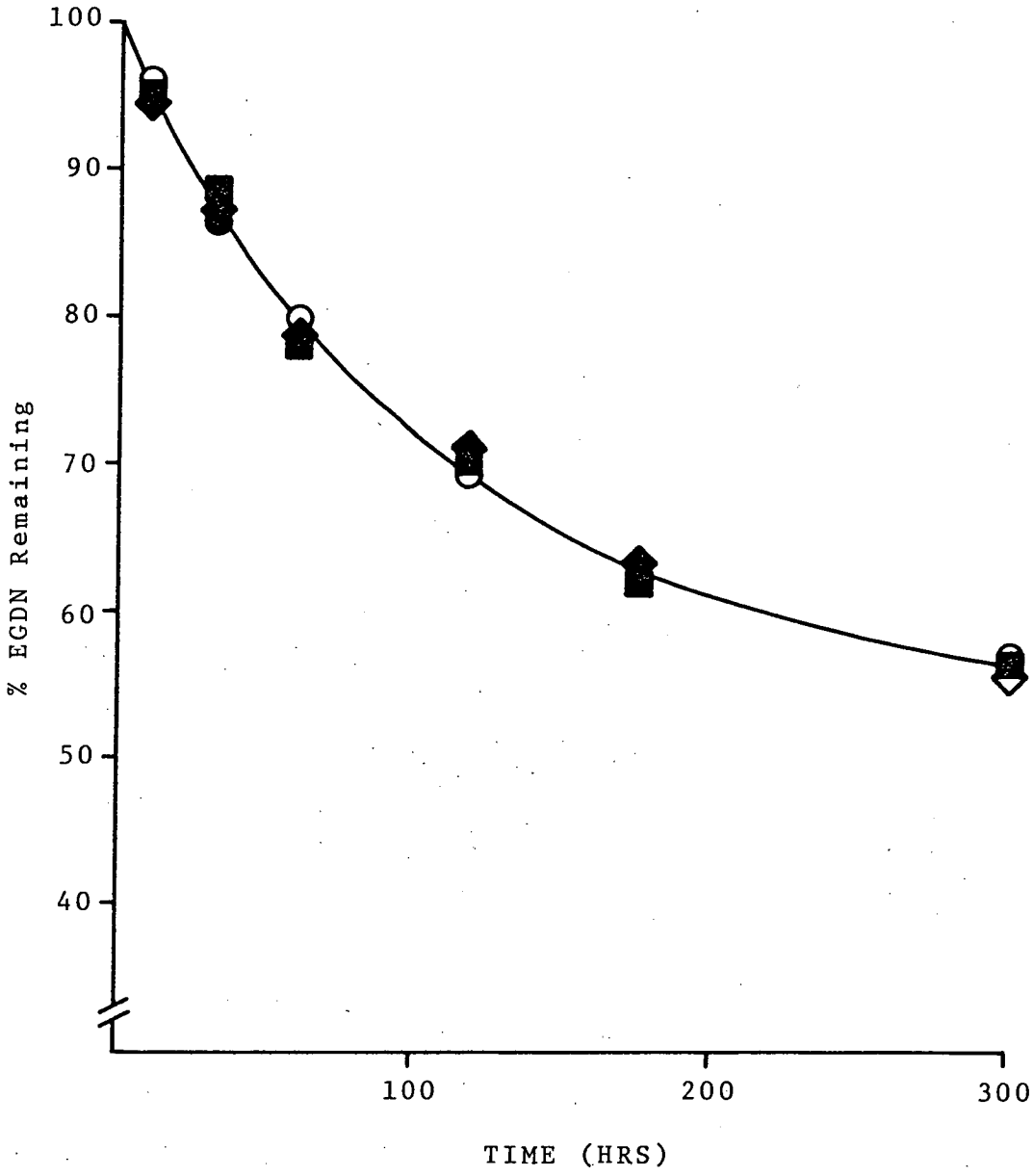


Figure 5.9 Percentage of ethylene glycol dinitrate (EGDN) remaining in solutions stored in plastic infusion bags for original concentrations of ethylene glycol dinitrate of 100 mcg/ml (open symbols) and 1000 mcg/ml (closed symbols).
○ ● water; □ ■ 5% dextrose in water;
◇ ◆ normal saline.

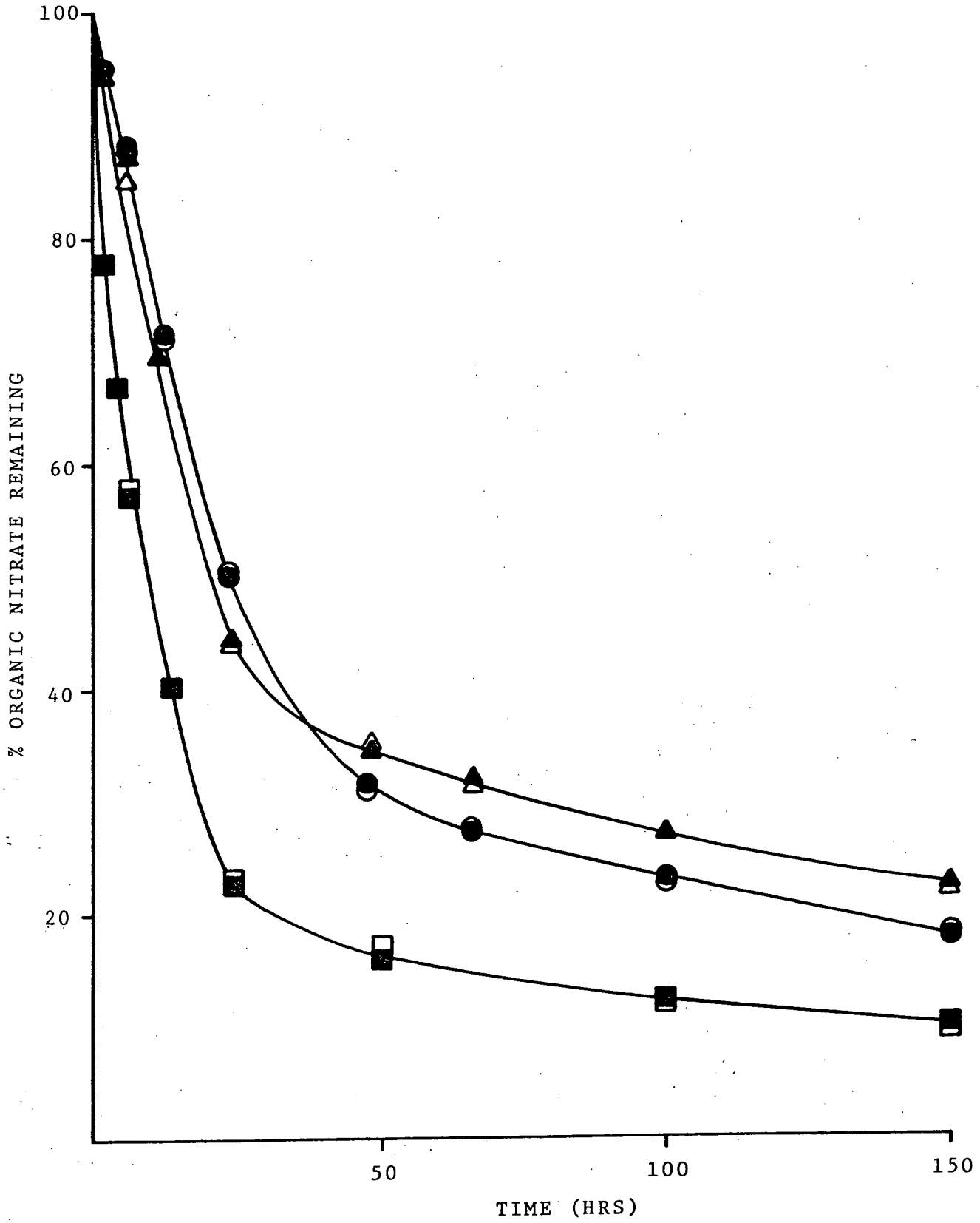


Figure 5.10 Percentage of nitroglycerin (□ ■) isosorbide dinitrate (○ ●) and ethylene glycol dinitrate (△ ▲) remaining in solutions stored in burettes of giving sets (open symbols, normal saline; closed symbols, 500 mcg/ml).

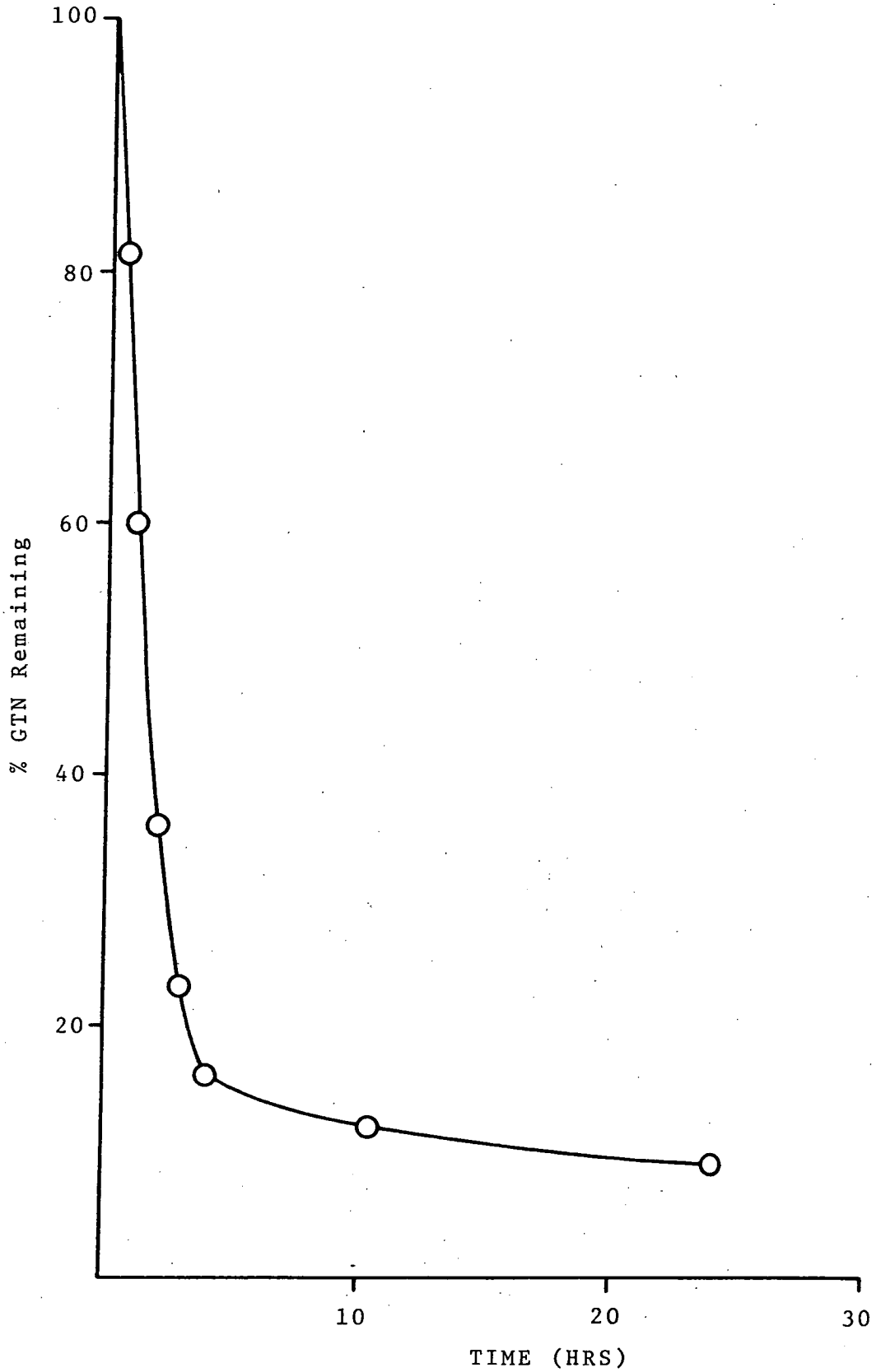


Figure 5.11 Percentage of nitroglycerin (GTN) (200 mcg/ml of water) remaining in solutions stored in plastic infusion tubing.

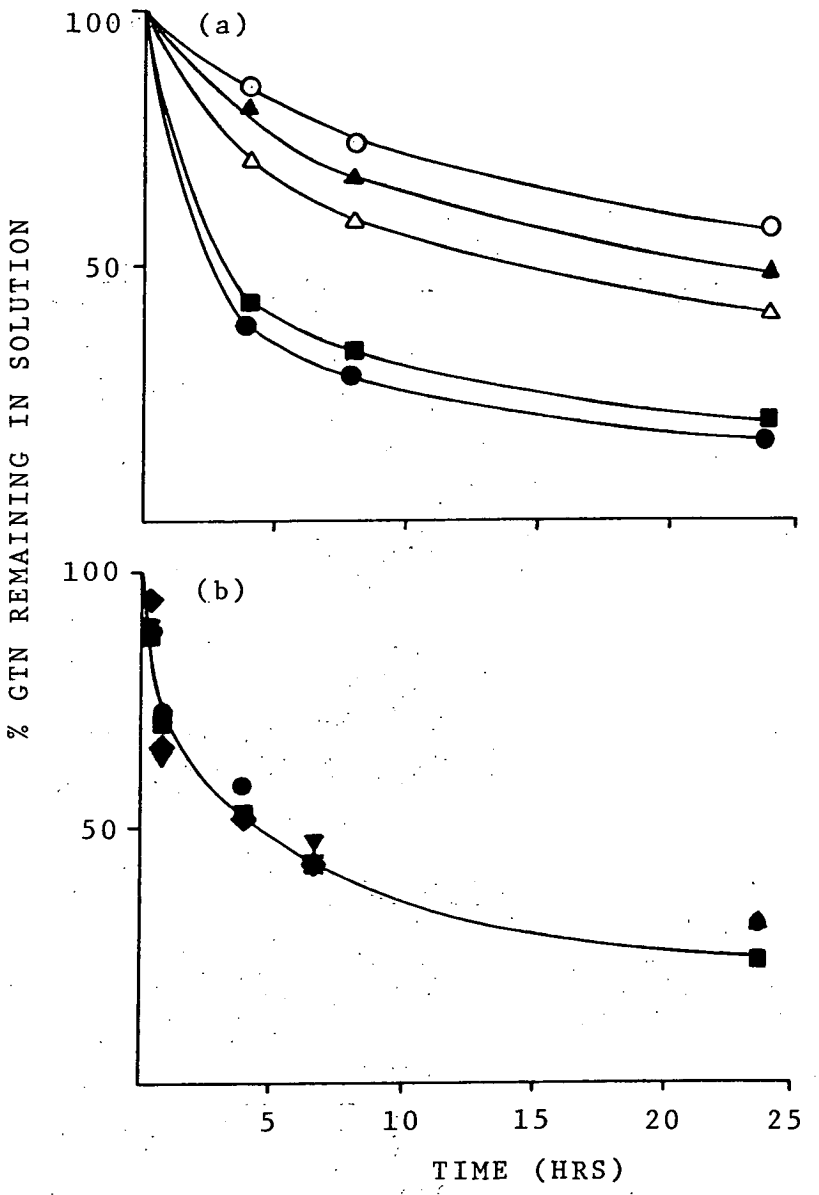


Figure 5.12 Effect of volume of nitroglycerin (GTN) solutions (200 mcg/ml water) stored in plastic infusion bags (a) and burettes (b) on the percentage of nitroglycerin remaining in solution. ▼ 10 ml; ● 50 ml; ■ 100 ml; ◆ 150 ml; △ 200 ml; ▲ 300ml; ○ 500 ml.

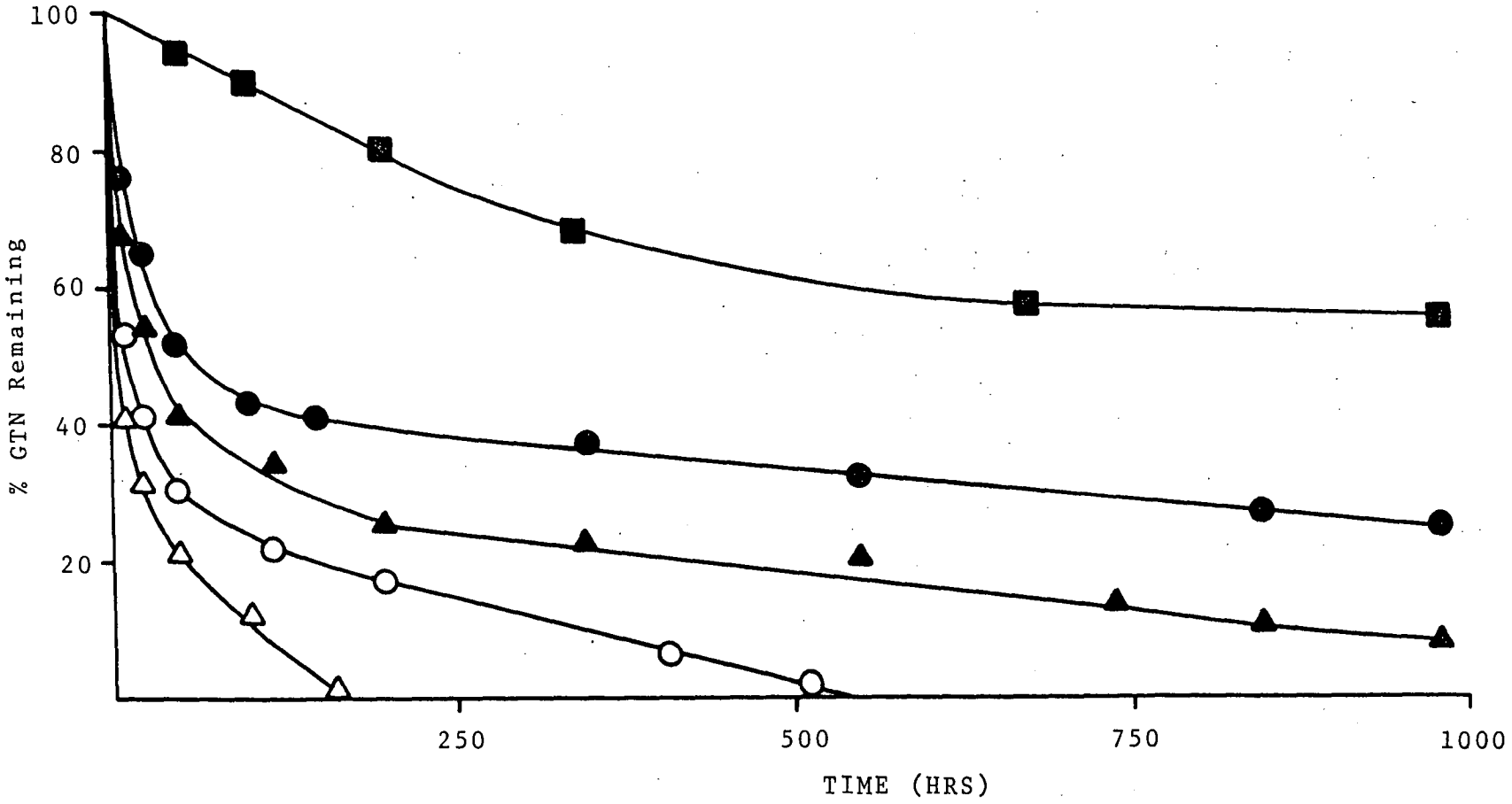


Figure 5.13 Effect of temperature on the percentage of nitroglycerin (GTN) remaining in solutions stored in plastic infusion bags. ■ 4°C; ● room temperature (20° - 24°C); ▲ 37°C; ○ 45°C; △ 60°C.

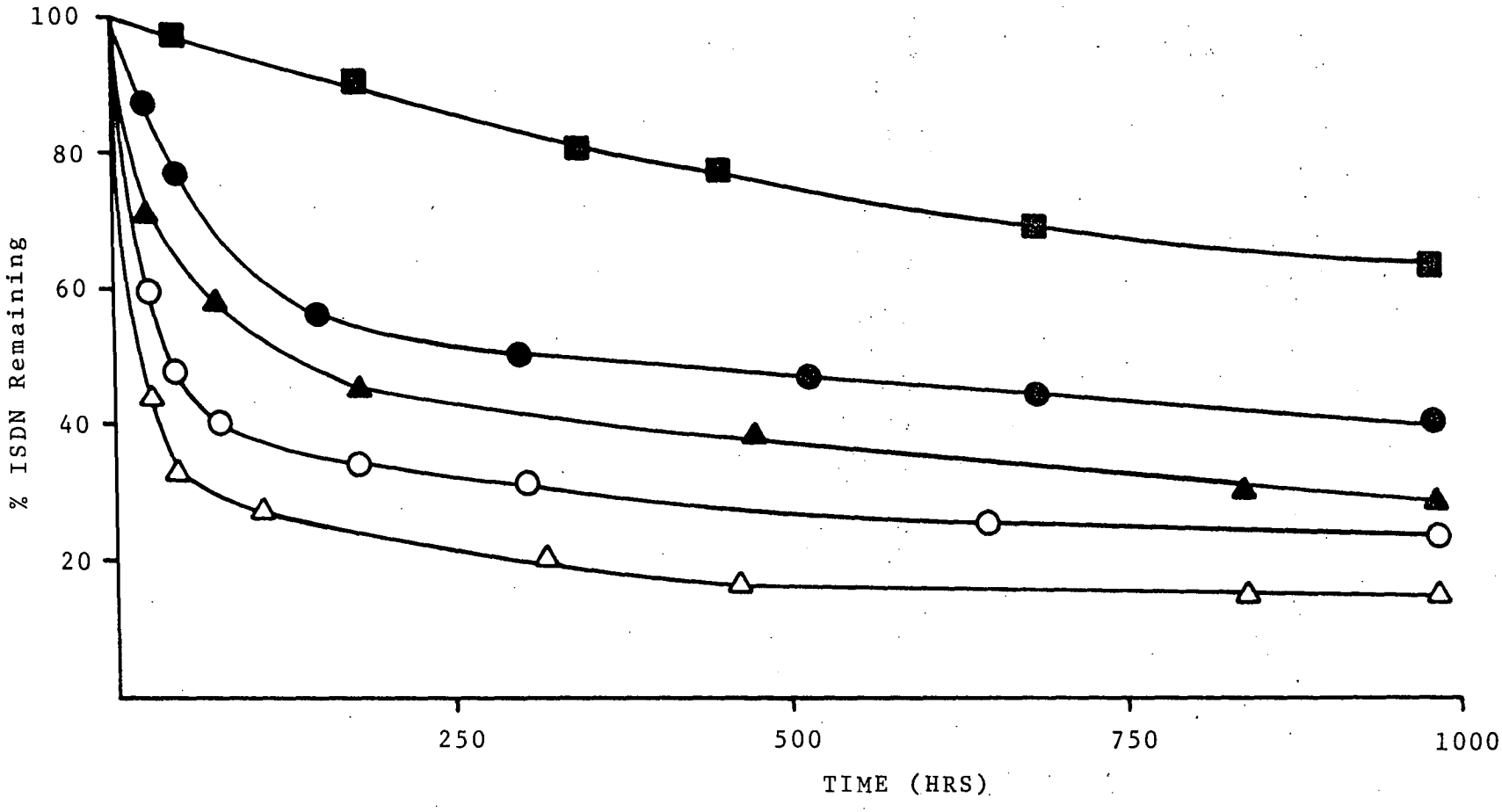


Figure 5.14 Effect of temperature on the percentage of isosorbide dinitrate (ISDN) remaining in solutions stored in plastic infusion bags. ■ 4°C; ● room temperature (20° - 24°C); ▲ 37°C; ○ 45°C; △ 60°C.

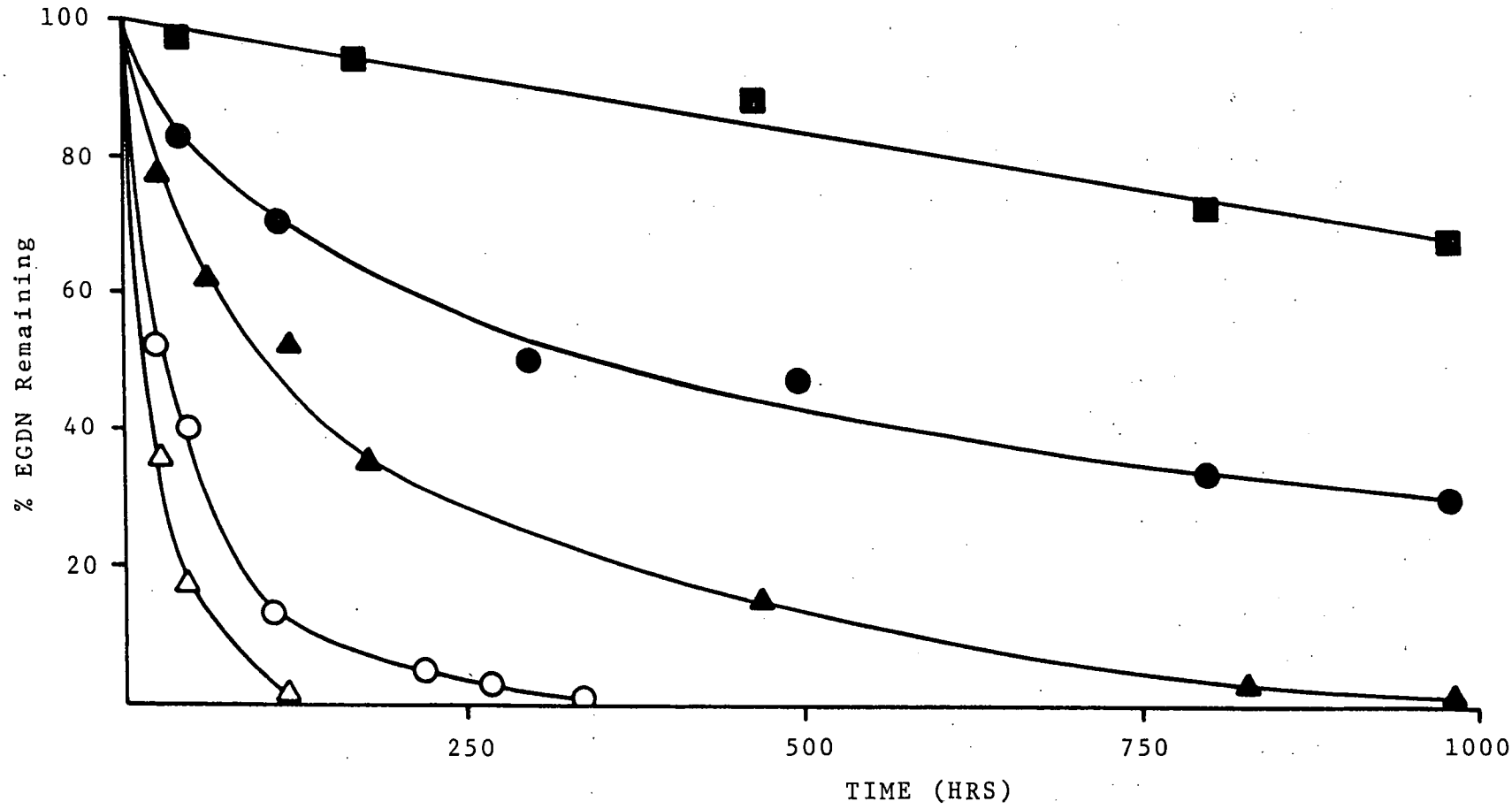


Figure 5.15 Effect of temperature on the percentage of ethylene glycol dinitrate (EGDN) remaining in solutions stored in plastic infusion bags. ■ 4°C; ● room temperature (20° - 24°C); ▲ 37°C; ○ 45°C; △ 60°C.

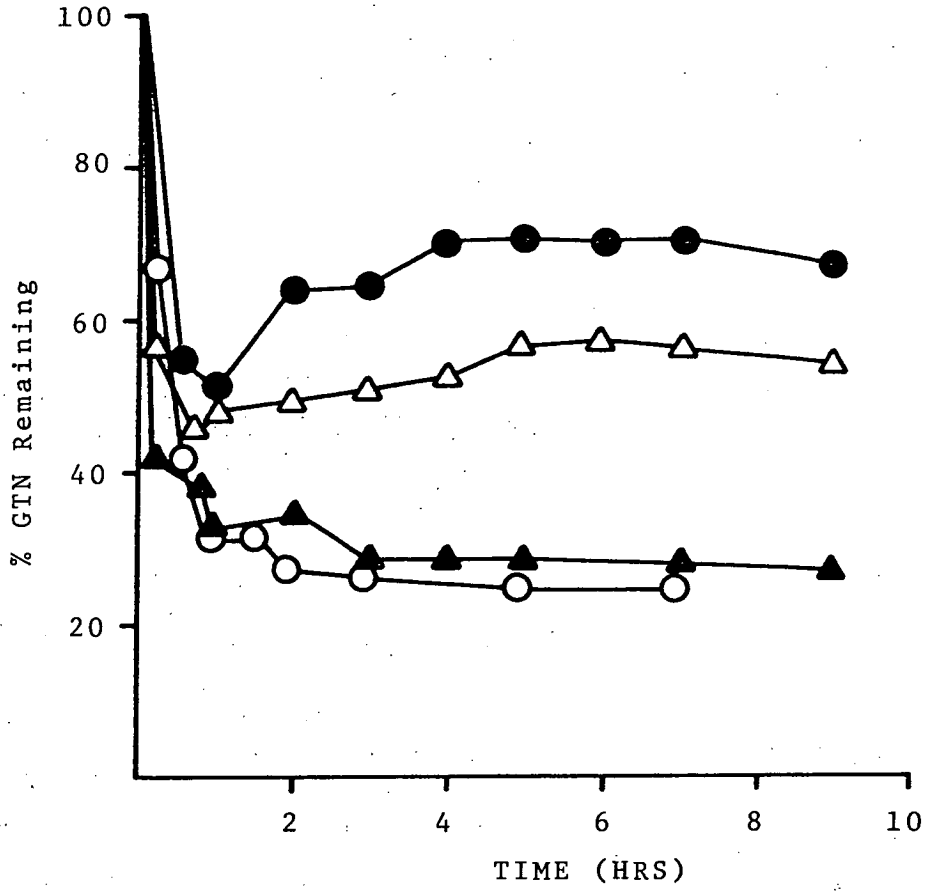


Figure 5.16 Effect of flow rate of the drug solution on the percentage of nitroglycerin remaining in solution after passage through plastic giving sets. ● 0.91 ml/min; △ 0.30 ml/min; ▲ 0.17 ml/min; ○ 0.07 ml/min.

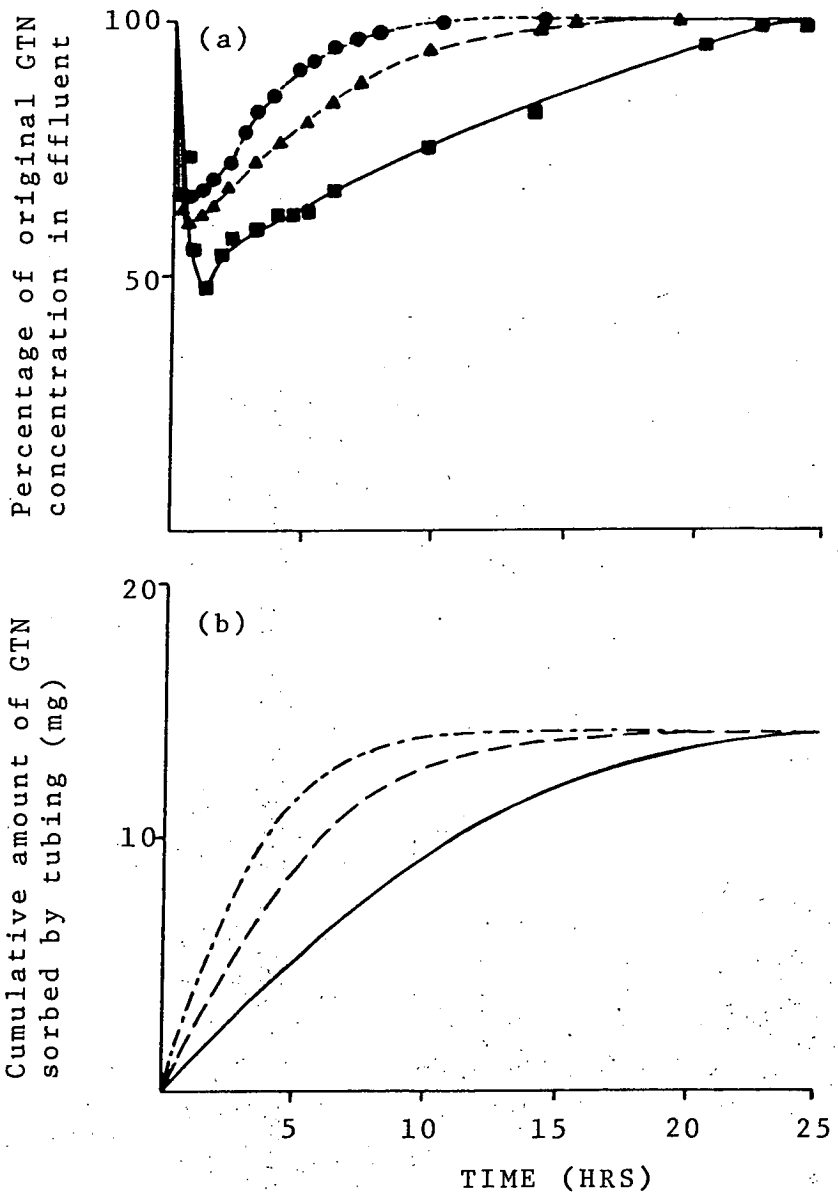


Figure 5.17 (a) Percentage of original nitroglycerin (GTN) concentration in the effluent for solutions infused from a glass infusion bottle through plastic infusion tubing at constant flow rates. — - — 0.75 ml/min; - - - 0.52 ml/min; —— 0.23 ml/min.

(b) Cumulative amount of nitroglycerin sorbed by the tubing for these flow rates.

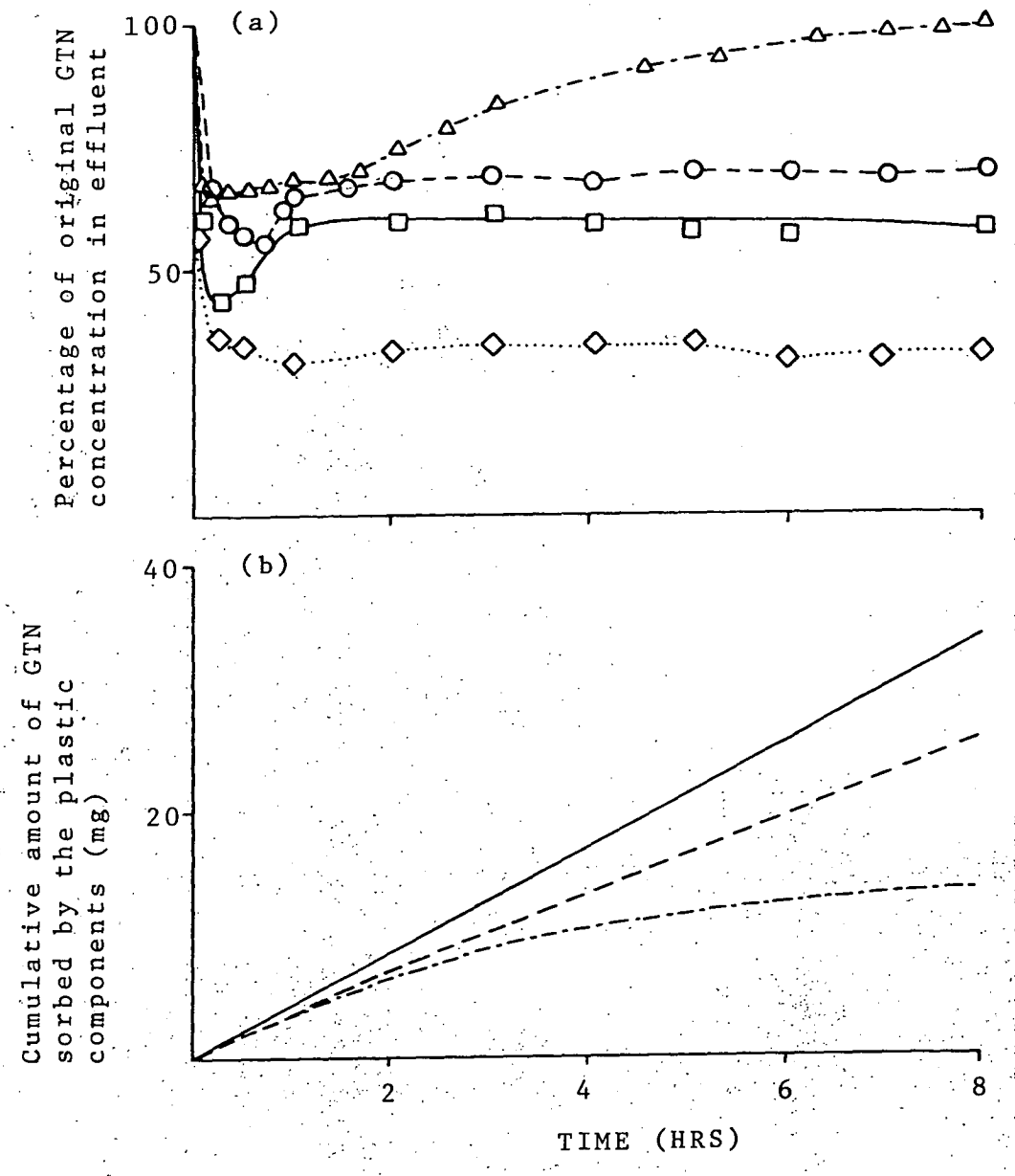


Figure 5.18 (a) Simulated infusions of nitroglycerin (GTN) at a constant flow rate (0.75 ml/min) using various infusion systems.

— - — - glass infusion bottle plus infusion tubing;

—— —— glass infusion bottle plus Buretrol giving set;

———— Viaflex plastic infusion bag plus Buretrol giving set;

..... Viaflex plastic infusion bag plus Buretrol giving set where nitroglycerin solution prepared in infusion bag 24 hrs before infusion.

(b) Cumulative amount of nitroglycerin sorbed by the plastic components of the infusion systems.

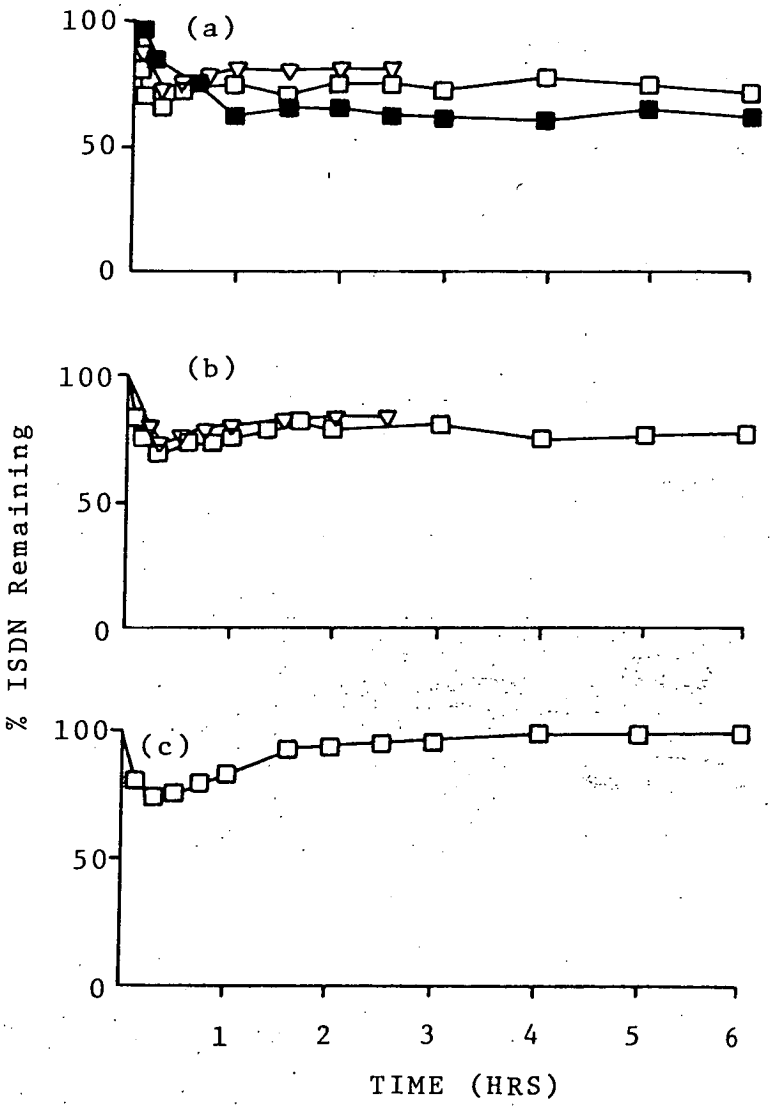


Figure 5.19 Percentage of original isosorbide dinitrate concentration (ISDN) in the effluent at various times for solutions infused using (a) infusion bag/giving set, (b) glass bottle/giving set and (c) glass bottle/infusion tubing. ▽ 1.60 ml/min; □ 0.73-0.78 ml/min; ■ 0.2 ml/min.

Table 5.3. Comparison of recoveries of nitroglycerin (GTN) and isosorbide dinitrate (ISDN) during infusion using glass bottle/plastic infusion sets and glass syringe/infusion pumps.

Drug	Infusion Time (hours)	Infusion Rate (ml/min)	Per Cent Drug Recovered	
			Plastic Infusion Sets	Glass Syringe Infusion Pump
GTN	8	0.07	28	95.2
	8	0.75	66	99.1
ISDN	6	0.75	70.1	100

Table 5.4. Loss of isosorbide dinitrate during infusions using different intravenous administration systems

INFUSION SYSTEM	INFUSION RATE ml/min.	PER CENT DRUG RECOVERED
Bag + Buretrol	0.20	63.3
	0.75	70.1
	1.60	77.1
Glass bottle + Buretrol	0.78	79.4
	1.60	81.4
Glass bottle + Tubing	0.73	91.5

infusion systems is plotted against time (results for the bag prepared 24hrs in advance are not included because more than 40mg of nitroglycerin was sorbed by the infusion bag before the infusion).

Recoveries, expressed as percentages of original strength, of nitroglycerin in the bulk samples collected during some infusions are shown in Table 5.3.

(b) Isosorbide Dinitrate

The results of similar experiments involving the loss of isosorbide dinitrate from aqueous solutions are presented in Figure 5.19 and Tables 5.3 and 5.4. Recoveries of isosorbide dinitrate after simulated infusions were greater than recoveries of nitroglycerin for a comparable flow rate.

5.3. KINETIC STUDIES WITH PLASTIC SHEETS AND TUBING

5.3.1. Sorption

The rate at which an organic nitrate was taken up by polyvinyl chloride was temperature dependent. However, the absolute amount of any one drug sorbed was the same for most temperatures (Figures 5.20 to 5.22).

Ethylene glycol dinitrate and nitroglycerin reached an

equilibrium between water and cellulose propionate. After 1 month isosorbide dinitrate had not reached an equilibrium between water and plastic. The profiles of uptake of these drugs are shown in Figure 5.23.

5.3.2. Permeation

The rates and extent of loss of nitroglycerin and ethylene glycol dinitrate from aqueous solutions in contact with polyvinyl chloride sheets at 45°C were greater in the diffusion cells in which the plastic sheets were not completely covered by metal (Figures 5.24 and 5.26). In cells where the plastic sheet was covered by metal, an equilibrium concentration of drug in solution was approached, however in those cells in which the plastic sheet was exposed to the air, a point was approached at which there was no drug left in solution. This effect occurred at a faster rate for ethylene glycol dinitrate at 45°C than for nitroglycerin at 45°C.

There were no differences in the rate or extent of loss of isosorbide dinitrate from aqueous solutions in contact with polyvinyl chloride sheets at 45°C when these sheets were either covered by metal or left uncovered (Figure 5.25).

5.3.3. Partition Coefficients

Octanol-water, hexane-water, polyvinyl chloride-water,

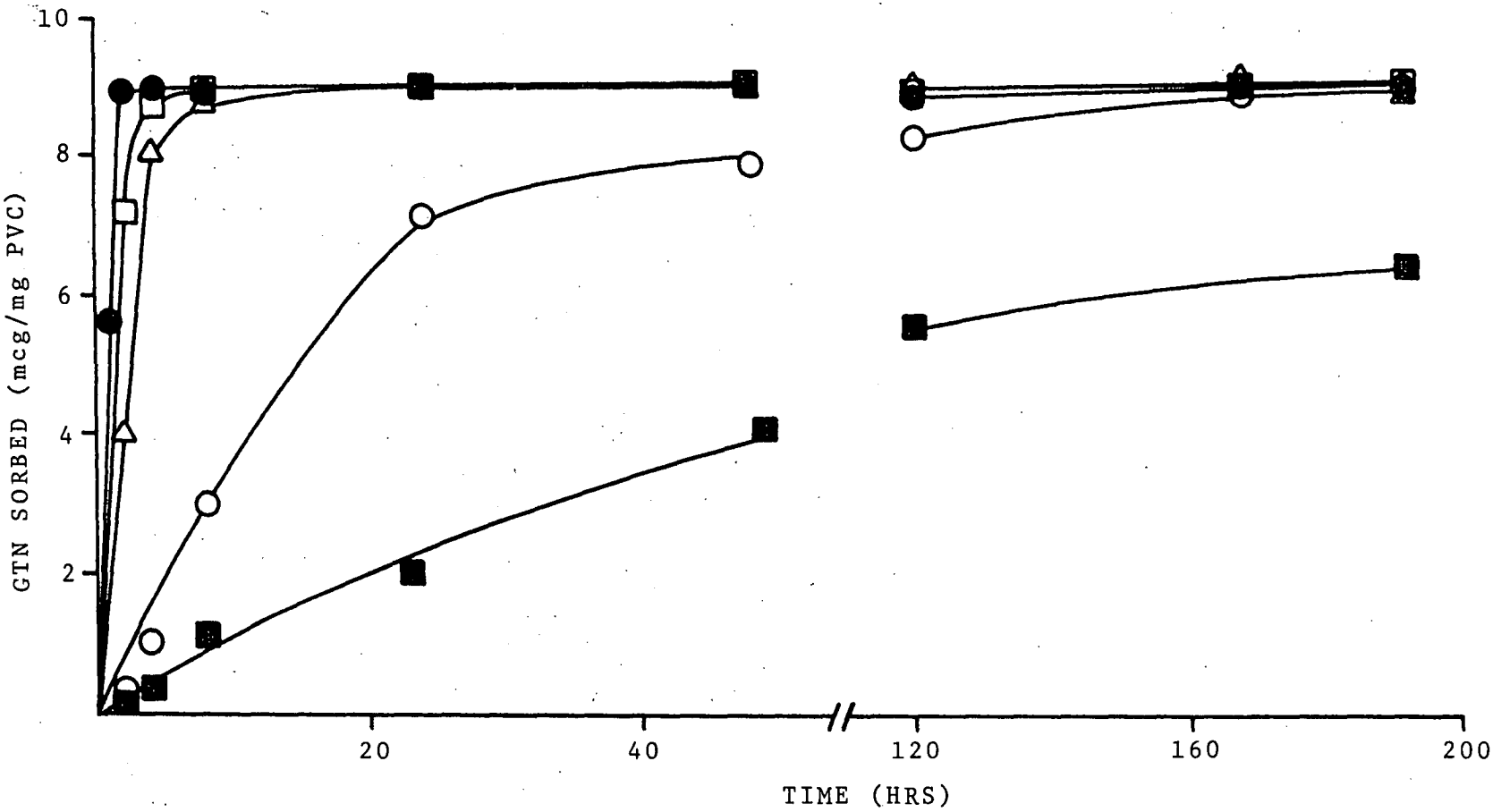


Figure 5.20 Uptake with time of nitroglycerin (GTN) by polyvinyl chloride (PVC) strips immersed in drug solution (400 mcg/ml water) at ■ 4°C; ○ room temperature (20° - 24°C); △ 37°C; □ 45°C; ● 60°C.

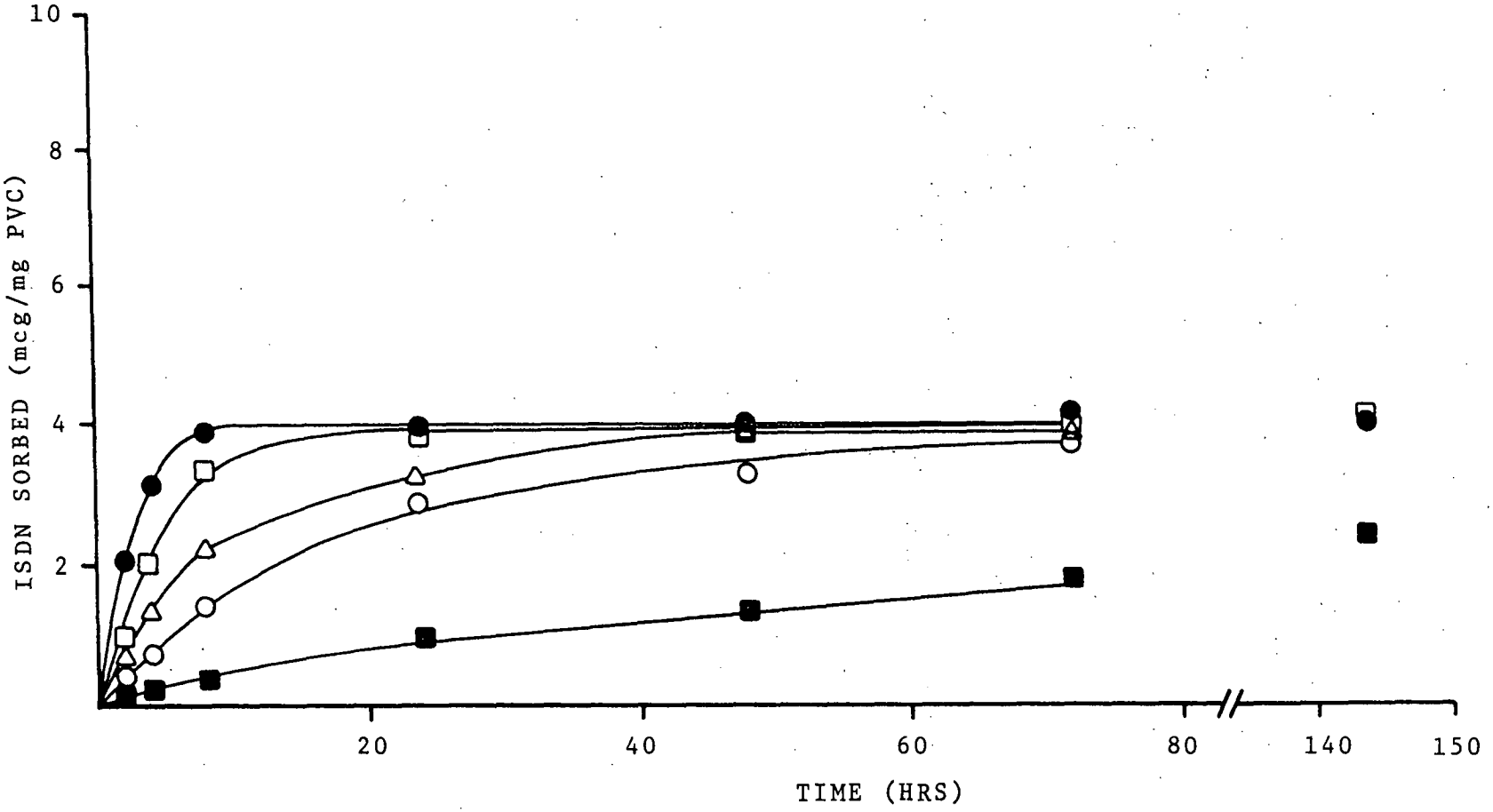


Figure 5.21 Uptake with time of isosorbide dinitrate (ISDN) by polyvinyl chloride (PVC) strips immersed in drug solution (275 mcg/ml water) at ■ 4°C; ○ room temperature (20°C - 24°C); △ 37°C; □ 45°C; ● 60°C.

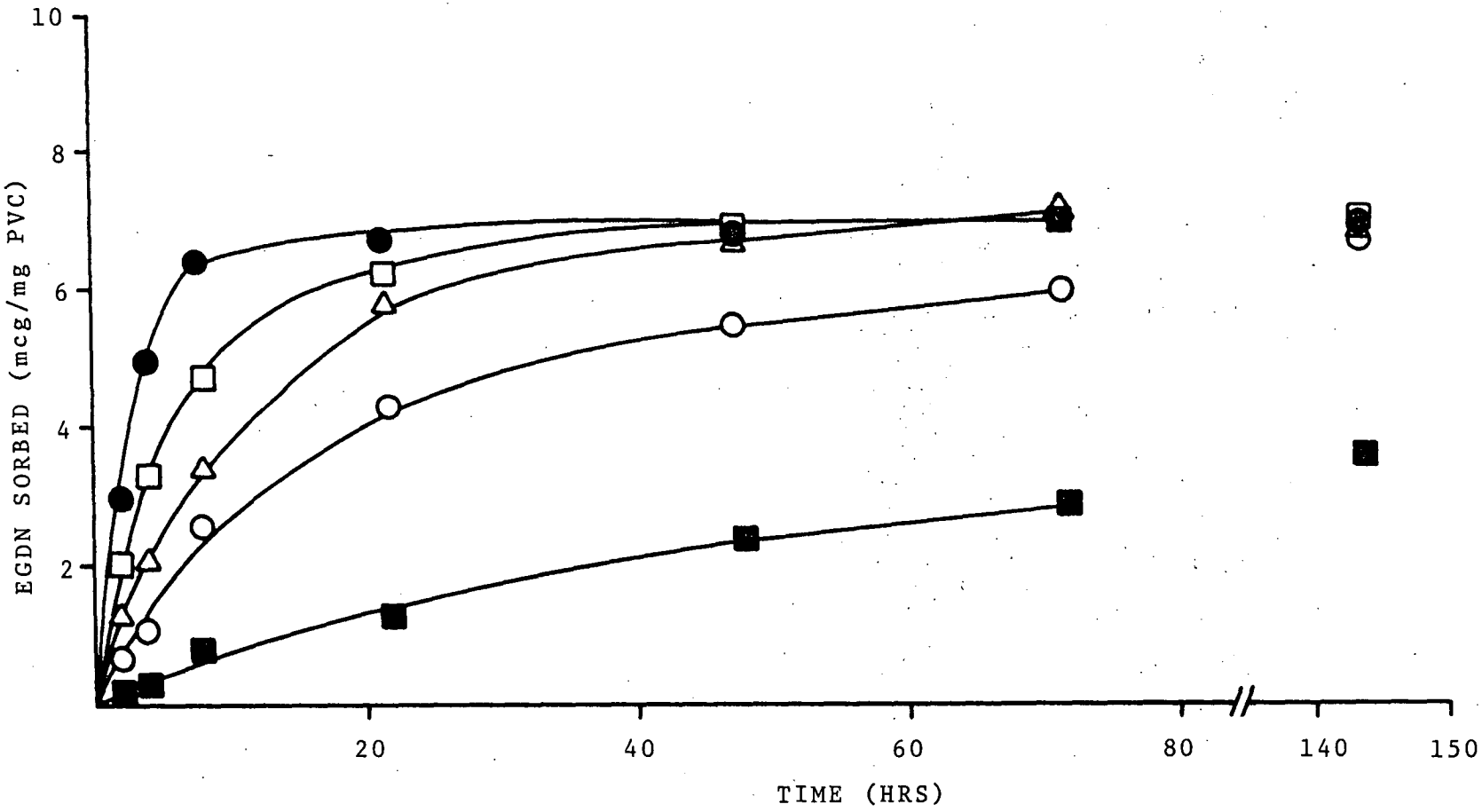


Figure 5.22 Uptake with time of ethylene glycol dinitrate (EGDN) by polyvinyl chloride (PVC) strips immersed in drug solution (500 mcg/ml water) at ■ 4°C; ○ room temperature (20°C - 24°C); △ 37°C; □ 45°C; ● 60°C.

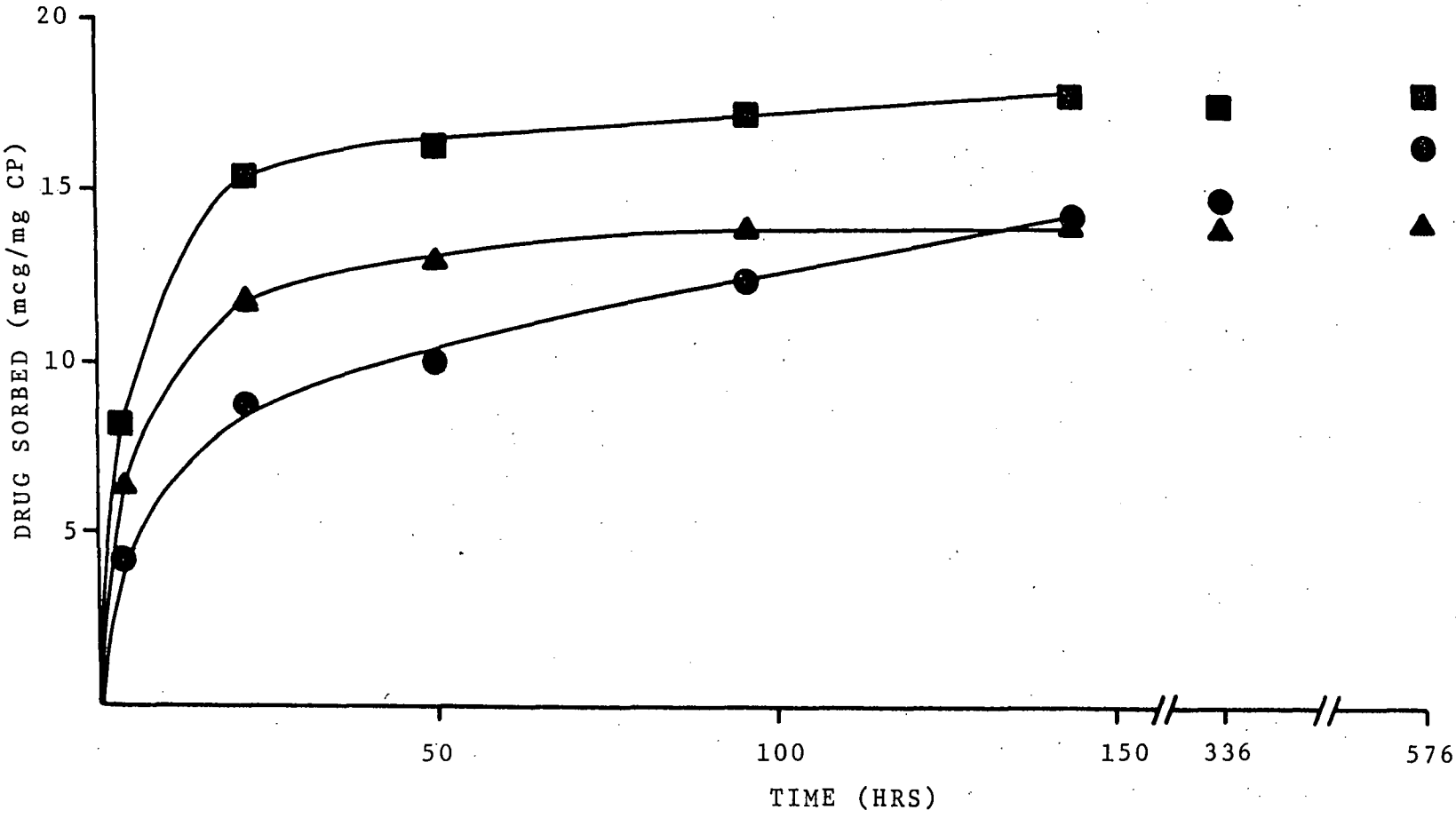


Figure 5.23 Uptake with time of nitroglycerin (■), isosorbide dinitrate (●) and ethylene glycol dinitrate (▲) by cellulose propionate (CP) strips immersed in drug solution (400 mcg/ml water) at room temperature.

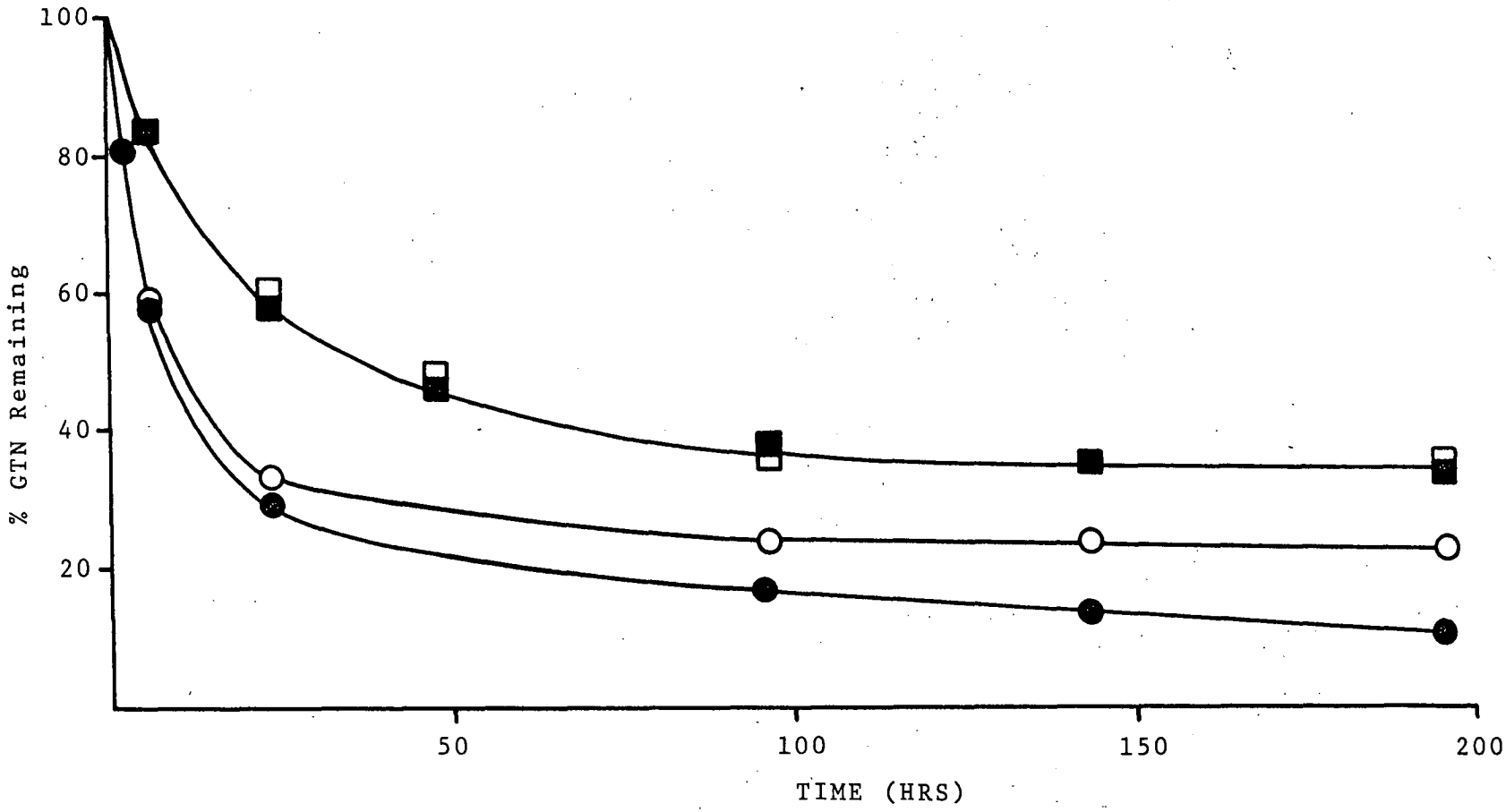


Figure 5.24 Percentage of nitroglycerin (GTN) remaining in solutions stored in contact with covered (open symbols) or uncovered (closed symbols) polyvinyl chloride sheets using a diffusion cell. □ ■ room temperature (20°- 24°C); ○ ● 45°C.

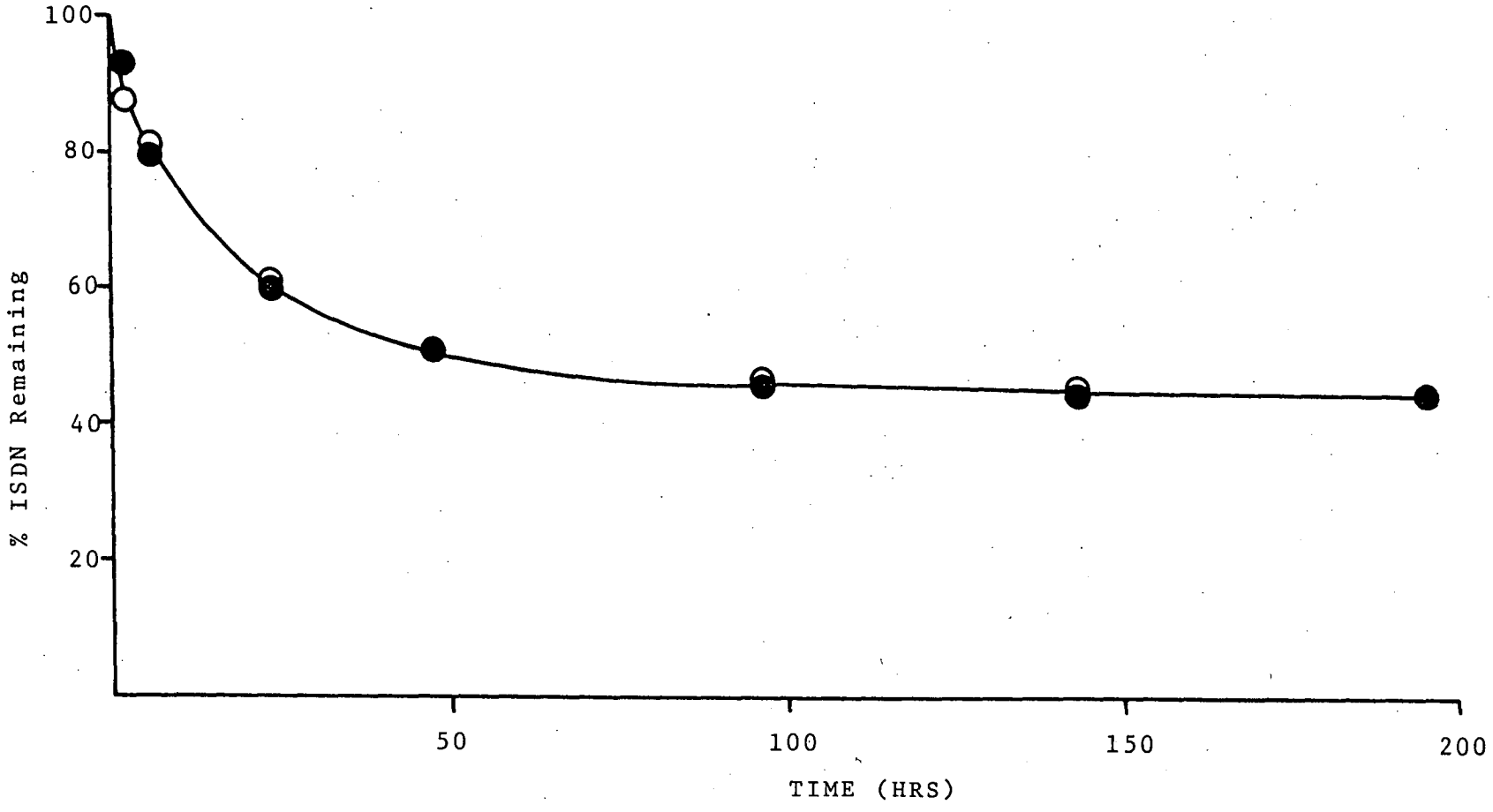


Figure 5.25 Percentage of isosorbide dinitrate (ISDN) remaining in solutions stored in contact with covered (O) or uncovered (●) polyvinyl chloride sheets using a diffusion cell at 45°C.

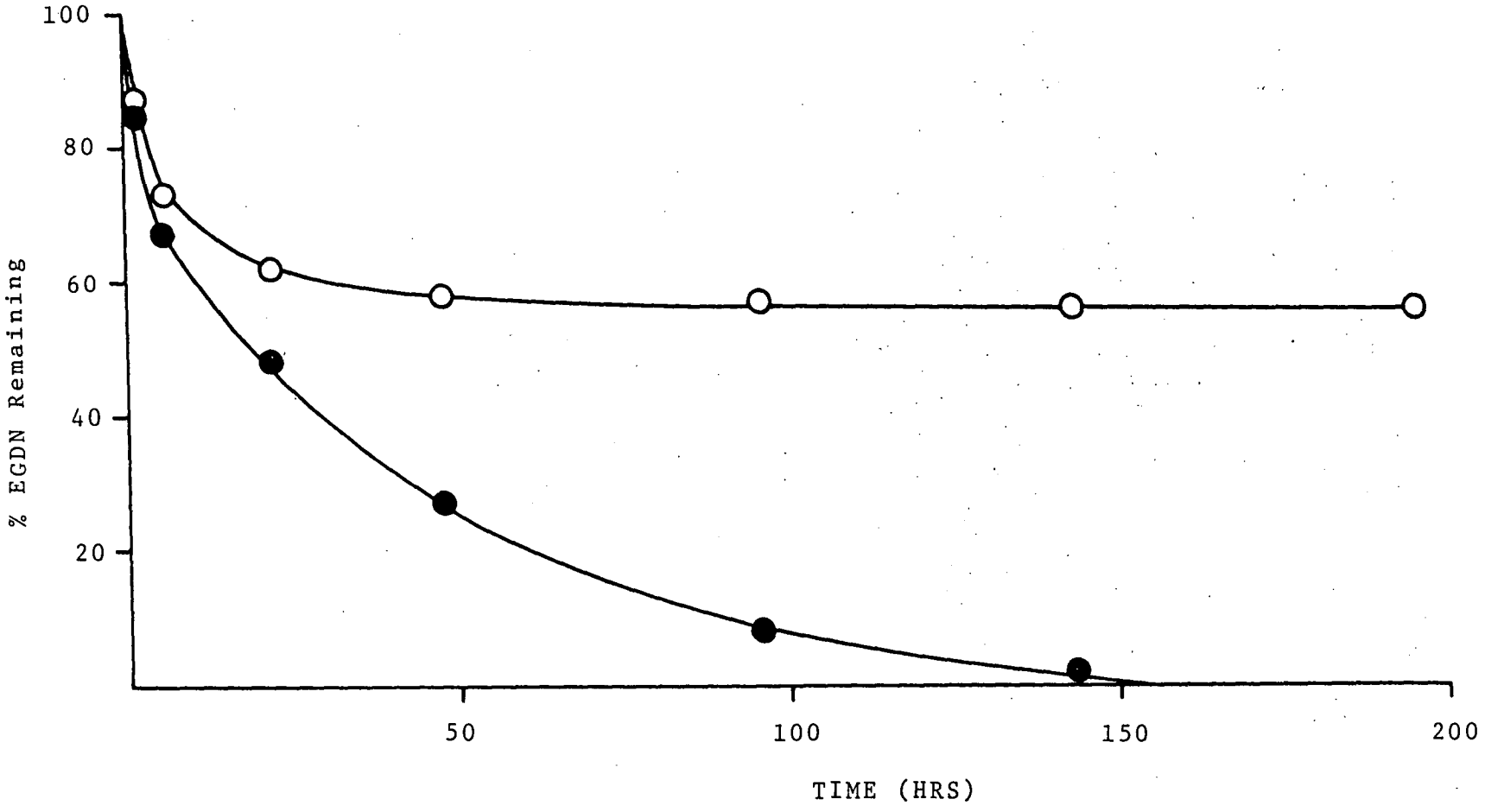


Figure 5.26 Percentage of ethylene glycol dinitrate (EGDN) remaining in solutions stored in contact with covered (O) or uncovered (●) polyvinyl chloride sheets using a diffusion cell at 45°C.

Table 5.5. Partition coefficients of nitroglycerin (GTN), isosorbide dinitrate (ISDN) and ethylene glycol dinitrate (EGDN)

	GTN	ISDN	EGDN
Octanol/water	41.8	20.6	14.6
Hexane/water	2.1	1.4	1.3
Polyvinyl chloride/water	115.2	38.7	31.2
Cellulose propionate/water	1118	-	130
High density polyethylene/water	0.3	< 0.3	< 0.3

cellulose propionate-water and high density polyethylene-water partition coefficients of the organic nitrates are presented in Table 5.5. The octanol-water and hexane-water partition coefficients of the organic nitrates are in the same rank order as the polyvinyl chloride-water and cellulose propionate-water partition coefficients.

The polyvinyl chloride-water partition coefficients of the three drugs were not dependent on temperature (Figures 5.20 to 5.22).

5.4. PREVENTION OF LOSS

5.4.1. Organic Nitrates

When solutions of nitroglycerin were infused from glass syringes through high density polyethylene tubing, the extent of loss was greatly reduced when compared to plastic giving sets. The loss was flow rate dependent, however, even at the very slow flow rate of 0.07 ml/min the loss was only about 5% and at 0.75 ml/min the loss was only 0.9% (Table 5.3).

No loss of isosorbide dinitrate occurred when infusing this drug from glass syringes through high density polyethylene tubing (Table 5.3).

5.4.2. Other Drugs

No loss of diazepam or chlormethiazole occurred when infusing these drugs from glass syringes through high density polyethylene tubing.

CHAPTER 6

DISCUSSION

6.1. ANALYTICAL METHODS

The kinetic assay of Fung et al (1973) for nitroglycerin in aqueous solutions is also suitable for the analysis of erythritol tetranitrate, mannitol hexanitrate, pentaerythritol tetranitrate and other poly-nitric esters bearing more than two nitrate groups per molecule (Yap et al, 1975a). Isosorbide dinitrate and ethylene glycol dinitrate, having only two nitrate groups per molecule, are unsuitable for analysis by this method. For convenience, as well as to detect any breakdown products of the parent drugs, the HPLC method was used. This method was used to complete initial studies involving nitroglycerin, and when shown to be precise for the analysis of isosorbide dinitrate and ethylene glycol dinitrate, was also used in studies involving interactions of those two drugs with plastic intravenous equipment.

6.2. STORAGE STUDIES

6.2.1. Formulation Factors

Nitroglycerin for intravenous infusion has been formulated in normal saline (Kaplan et al, 1976) and in 5% dextrose in

water (Chiche et al, 1979). The lack of effect of normal saline and 5% dextrose on the disappearance rate of nitroglycerin from aqueous solutions stored in plastic infusion bags (Figure 5.7) is consistent with the previous report of Sturek et al (1978).

The concentrations of nitroglycerin and isosorbide dinitrate used for intravenous infusions vary considerably. Concentrations of 32 mcg/ml (Kaplan et al, 1976), 60 mcg/ml (Chiche et al, 1979), 100 mcg/ml (Christensson et al, 1969), 120 and 240 mcg/ml (Hill et al, 1981) and 400 mcg/ml (Flaherty et al, 1975) have been used in nitroglycerin infusions, while for isosorbide dinitrate infusions, concentrations of 50 mcg/ml (Taylor et al, 1980) and 200 mcg/ml (Distante et al, 1979) have been used.

In the current work the fraction of nitroglycerin and isosorbide dinitrate, as well as ethylene glycol dinitrate, sorbed from solution by the infusion bags has been shown to be independent of the concentration of drugs used (Figures 5.7 to 5.9). The fractions of vitamin A and methohexital sorbed from solutions by Viaflex plastic infusion bags also appear to be independent of concentration, whereas for warfarin, the fraction sorbed decreases with increasing warfarin concentrations (Moorhatch & Chiou, 1974a). In a recent publication, Parker et al (1979) reported that the percentage loss of diazepam from infusion bags was a function of concentration. Their results could also be

explained, in part, by differences in the surface areas of plastic per unit volume of solution for the two concentrations used.

The volume of nitroglycerin solution in a plastic infusion bag has been shown to influence the fractional loss of nitroglycerin from solution (Figure 5.12a). As the volume of the nitroglycerin solution in the plastic infusion bag decreases during infusions, the fraction of drug sorbed increases because the surface area of plastic to volume of solution ratio increases as the volume of solution in the bag is decreased (e.g. during nitroglycerin infusion) due to the "collapsing" of the bag. Thus, the concentration of nitroglycerin in the smaller volumes decreases to a greater extent with time than for the larger volumes. This means that towards the end of an infusion, the fractional loss of nitroglycerin is relatively higher than at the beginning because not only is drug still being lost to the burette and tubing of the giving set, but a greater rate of loss of drug to the bag is occurring.

Polack et al (1970) and Roberts et al (1979) have also shown that the disappearance of some solutes from plastic containers during storage is a function of the surface area of plastic per unit volume of solution.

For all volumes of solution stored in the burette, the surface area of plastic per unit volume of solution is

constant when the contribution of the base of the burette (a high density plastic) to the total surface area is neglected. Consequently, the fraction of nitroglycerin remaining in aqueous solutions stored in the burettes for various times appears to be independent of the volume of solution in the burette (Figure 5.12b).

It has been suggested by Fung (1978) and McNiff et al (1979) that some discrepancies have appeared in the literature concerning the stability of nitroglycerin solutions for intravenous use. In the present work, the loss of nitroglycerin from aqueous solutions stored in glass containers (with metal screw caps) over 96hrs was minimal (<1%), consistent with the observations of McNiff et al (1979) and Baaske et al (1980). Indeed, even after storage for up to 1000hrs, there was only minimal loss of nitroglycerin (<2%) and this was probably due to hydrolysis. Other studies (Sturek et al, 1978; Ludwig & Ueda, 1978) showed some loss of nitroglycerin stored in glass containers. In the study of Ludwig & Ueda (1978), loss probably arose from interaction of nitroglycerin with the rubber/plastic components (e.g. bung, administration set) of the bottles which were used.

6.2.2. Temperature

At the elevated temperatures of accelerated stability testing, nitroglycerin, isosorbide dinitrate and ethylene

glycol dinitrate were rapidly lost from their aqueous solutions when stored in polyvinyl chloride infusion bags (Figures 5.13 to 5.15). For nitroglycerin and isosorbide dinitrate, the clinically used drugs, loss was very much slower at 4°C than at room temperature or above. This temperature-dependent loss of nitroglycerin has also been reported by McNiff et al (1979) and Baaske et al (1980). Although less than 10% of the original concentration of these two drugs is lost during storage in infusion bags for 24hrs at 4°C, the temperature of the solution has to be increased to about room temperature before infusions can be given. At this higher temperature significant amounts of nitroglycerin and isosorbide dinitrate are lost from solution. Thus, keeping the bags at 4°C only temporarily reduces the problem of loss due to sorption.

At elevated temperatures, hydrolysis of the organic nitrates appeared to play no part in the loss of these drugs. High-pressure liquid chromatographs did not show the presence of any lower esters of any of the three organic nitrates tested. This is consistent with literature reports on the hydrolysis of ethylene glycol dinitrate in aqueous solution at 60°C (Urbanski, 1965). Over the time of the experiments using plastic bags at 60°C (1000 hours), 0.067% of the ethylene glycol dinitrate would have been expected to be hydrolyzed according to the figures of Urbanski (1965). The same author presents data to suggest that hydrolysis of nitroglycerin in aqueous

solution at 60°C proceeds at a rate much slower than that for ethylene glycol dinitrate.

6.2.3. Light

The finding that room light had no effect on the loss of nitroglycerin from solutions stored in plastic bags has also been reported by Sturek et al (1978) and Ludwig and Ueda (1978).

6.3. DYNAMICS OF ORGANIC NITRATE - PLASTIC INTERACTION

6.3.1. Sorption and Permeation

The polyvinyl chloride-water partition coefficient obtained for nitroglycerin is consistent with that reported previously by Yuen et al (1979). These workers also found that the partition coefficient is independent of initial concentration of drug in solution. In the current work, the polyvinyl chloride-water partition coefficients (K) calculated from sorption studies are in the rank order K(GTN) > K(ISDN) > K(EGDN) and this parallels both the octanol-water partition coefficients and hexane-water partition coefficients for these drugs (Table 5.5). Other workers have found a similar rank order of the cottonseed oil-water and corn oil-water partition coefficients for nitroglycerin and isosorbide dinitrate (Table 6.1). The cottonseed oil-water partition coefficients for

Table 6.1. Partition Coefficients of Nitroglycerin (GTN) and Isosorbide Dinitrate (ISDN)

Drug	Partition Coefficient				
	Octanol-water	Hexane-water	Cottonseed oil-water	Corn oil-water	PVC-water
GTN	41.8	2.1	115[a]	77[b]	115.2
ISDN	20.6	1.4	41[a]	28[b]	38.7

(a) Needleman and Johnson (1975).

(b) Levy (1970).

Table 6.2. Half-times for sorption of organic nitrates by polyvinyl chloride sheets.

	Half-time (hours) Temperature (°C)						Activation energy (Kcal/mole) ± S.D.
	4	22	30	37	45	60	
Nitroglycerin	61	13	3.2[a]	2	1.2	1	13.9 ± 0.31
Isosorbide dinitrate	100	14	-	6.5	4	2	12.1 ± 0.75
Ethylene glycol dinitrate	130	16	-	8.5	4.5	2.5	12.3 ± 0.16

(a) Yuen et al (1979).

nitroglycerin and isosorbide dinitrate determined by Needleman and Johnson (1975) are in very close agreement with the polyvinyl chloride-water partition coefficients found in the current work (Table 6.1).

No attempt was made to evaluate the reasons for the differences in the cottonseed oil-water, polyvinyl chloride-water, octanol-water and hexane-water partition coefficients because of the different natures of the binary systems in relation to hydrogen bonding and water content of the solvents. Polyvinyl chloride contains anti-oxidants, plasticizers and preservatives (Autian, 1971) and cottonseed oil contains membrane fragments with many -NH and -OH groups (Leo et al, 1971), all of which could play a role in the binding of the organic nitrates.

Studies of the sorption of the organic nitrates by cellulose propionate sheets showed that only ethylene glycol dinitrate and nitroglycerin reached equilibrium between water and plastic after 1 month (Figure 5.23). The larger plastic-water partition coefficients for the drugs associated with cellulose propionate compared to polyvinyl chloride (Table 5.4) are due to the differences in the nature of the plastics. The slower rate of uptake of isosorbide dinitrate compared to ethylene glycol dinitrate and nitroglycerin is probably due to the drug diffusing more slowly through the cellulose propionate. The large plastic-water partition coefficients associated with

cellulose propionate reflect the more rapid rate of loss of ethylene glycol dinitrate and nitroglycerin from solutions stored in intact burettes of giving sets than from solutions stored in intact polyvinyl chloride infusion bags.

Permeation studies at 45°C showed that nitroglycerin and ethylene glycol dinitrate are lost to the environment. However, there was no apparent loss of nitroglycerin to the environment when the experiments were performed at room temperature (some loss may possibly become apparent over much longer storage times) (Figures 5.24 to 5.26). This increased loss at higher temperatures is probably due to an increase in the vapour pressure of nitroglycerin and ethylene glycol dinitrate when the ambient temperature is raised from 20°C to 45°C (a twenty fold increase for nitroglycerin and a ten fold increase for ethylene glycol dinitrate) (Urbanski, 1965). It is unlikely that these differences result from chemical decomposition of the drugs in the plastic. Decomposition of the organic nitrates in the plastic would be expected to be the same in sheets exposed to the environment and in sheets protected by a metal "barrier".

Isosorbide dinitrate was lost from solution at the same rate and to the same extent for both covered and uncovered plastic sheets (Figure 5.25). Although no information about the vapour pressure of isosorbide dinitrate is

available in the literature, it is significant at room temperature (C. Fitzmaurice, personal communication) due to the semi-polar bond of its nitro groups, a characteristic of the other organic nitrates (Urbanski, 1965). If it has a lower vapour pressure than nitroglycerin then the reduced loss of isosorbide dinitrate at elevated temperatures compared to the other organic nitrates under investigation is consistent with the results obtained.

6.3.2. Models

It was not the purpose of the current work to evaluate different mathematical models with regard to their applicability in describing the loss of organic nitrates from aqueous solutions stored in or being infused through plastic intravenous infusion equipment. However, work in this laboratory has shown that the results obtained for the loss of nitroglycerin from solutions stored in polyvinyl chloride infusion bags were better described by a diffusion model (Section 2.1.2. (b)), than by a two compartment model (Section 2.1.2. (a)).

Yuen et al (1979) also obtained good agreement between their actual results and predictions based on a diffusion model when examining the uptake of nitroglycerin from aqueous solution by polyvinyl chloride strips immersed in drug solution. These workers presented results which showed that the time for 50% sorption of nitroglycerin at

30°C was 3.2 hours. This time is compared to times for 50% sorption of nitroglycerin obtained from sorption studies in the current work at various temperatures in Table 6.2. It can be seen that the results of Yuen et al (1979) are consistent with results for the current work. Half-times for sorption of isosorbide dinitrate and ethylene glycol dinitrate are included in Table 6.2. These two organic nitrates appear to diffuse in the plastic more slowly than nitroglycerin probably as a result of subtle differences in their molecular structures.

The activation energies for diffusion for the three organic nitrates determined from the Arrhenius relationship were found not to be statistically significantly different. Autian (1971) suggested that the activation energy for diffusion of solutes in plastics corresponds to (1) the energy needed to move polymer chains sufficiently apart to create a "hole" and (2) the energy needed to move the diffusing molecule into the "hole". As the activation energies for the three organic nitrates are similar, it is unlikely that the nature of interaction of individual molecules with the plastic is determining the observed activation energies. Instead, the activation energies probably result from changes in the mobility of the polymer chains (in the polyvinyl chloride film) with temperature.

Yuen et al (1979) suggested that absorption of nitroglycerin was the major source of loss of drug, however

they could not discount the possibility of adsorption of the drug on the surface of the plastic. Consequently Sokoloski et al (1980) showed that a first-order adsorptive process was responsible for a rapid initial loss of nitroglycerin on polyvinyl chloride. The data of these workers conformed to Polanyi adsorption potential theory which has been described by Schenz and Manes (1975).

All of the above work was, however, performed using isolated polyvinyl chloride sheets fully immersed in aqueous drug solution. From the experimental data in the current work showing that loss of nitroglycerin from solutions stored in intact plastic bags still occurs after 1000hrs and that vaporization of nitroglycerin could play a part in the loss of this drug from aqueous solutions stored in intact polyvinyl chloride infusion bags, a combination of the Polanyi and diffusion models may still not accurately describe the loss of nitroglycerin.

6.4. SIMULATED INFUSIONS

6.4.1. Infusions Simulating Clinical Studies

(a) Nitroglycerin

The rate of infusion of nitroglycerin to patients suffering acute myocardial infarction generally starts at about 10-20 mcg/min and is then increased in increments of 10-20

mcg/min until a desired haemodynamic (fall in systemic pressure) or clinical (relief of chest pain) response occurs. Depending on the initial concentration of nitroglycerin in the infusion bag, initial flow rates associated with these doses have been in the range of about 0.04 ml/min (Hill et al, 1981) to 0.3 ml/min (Flaherty et al, 1975) with final infusion rates in the range of 2 ml/min (Hill et al, 1981) to 5 ml/min (Flaherty et al, 1975). Thus, as the loss of nitroglycerin during simulated infusions is flow rate dependent (Figure 5.16), the extent of loss actually occurring in the clinical situation will vary widely.

Results verifying the flow rate dependent loss of nitroglycerin (Figure 5.16) have recently been provided by Baaske et al (1980) and Christiansen et al (1980). The former workers also studied the loss of nitroglycerin associated with a flow rate of 2 ml/min and showed that about 30% of the original nitroglycerin is lost during a 4hr infusion. These workers not only used Buretrol giving sets in their study but also seven other commercially available sets. All sets tested produced similar results.

(b) Isosorbide Dinitrate

The loss of isosorbide dinitrate during infusion through plastic giving sets is also flow rate dependent (Figure 5.19). The extent of loss for any comparable flow rate is,

however, much less than observed for nitroglycerin. The extent of loss may be important in clinical studies in which isosorbide dinitrate has been infused. Distante et al (1979) infused isosorbide dinitrate during the treatment of angina at rest and claimed that this treatment was effective. If these workers used plastic infusion equipment to infuse isosorbide dinitrate, then some loss of the drug would probably have occurred. The rate of infusion of 0.1-0.3 ml/min used by Distante et al (1979) resulted, in the present study, in a recovery of only 63% when infused from a plastic bag through a plastic giving set and a recovery of only 70% when infused at a rate of 0.75 ml/min.

6.4.2. Infusion Tubing Effluent Concentration-Time Profiles

(a) Nitroglycerin

Greatest loss (i.e. smallest concentrations of nitroglycerin in the effluent) are observed for the slowest flow rates where the contact time of a given volume of solution with plastic is greatest. The times for the minimum concentrations of nitroglycerin in the effluent reflect the time required for the solution in the tubing at the beginning of the infusion to pass through the tubing. Subsequent solution entering the tubing from the glass infusion bottle will have a higher concentration of nitroglycerin in its effluent due to significant sorption

of nitroglycerin from the initial solution by the tubing. Since the tubing contains approximately 8ml of solution, times of about 10, 15 and 35min would be anticipated for flow rates of 0.75, 0.52 and 0.23 ml/min respectively. The observed times for minimum concentrations of nitroglycerin in the effluent (Figure 5.17a) are of similar magnitude to these values for corresponding flow rates. For all flow rates, the concentration of nitroglycerin in the effluent eventually returns to the original concentration of nitroglycerin in the infusion bottle (Figure 5.17a). Loss of nitroglycerin during these infusions therefore arises solely from sorption by the plastic tubing. If decomposition, adsorption to the glass infusion bottle or loss to the external (atmosphere) environment had occurred, the concentration of nitroglycerin in the effluent for long times (i.e. steady-state) would not approach the original nitroglycerin concentration in the glass infusion bottles. Consistent with an equilibrium sorption process, the total cumulative amount of nitroglycerin sorbed by the plastic infusion tubing is independent of flow rate (Figure 5.17b).

Since the sorption of nitroglycerin by the plastic infusion tubing is an equilibrium process, accurate delivery of known amounts of nitroglycerin to the patient could theoretically be achieved by pretreating the tubing before an infusion. For instance, the tubing could be flushed with solution from the attached glass infusion bottle at a rapid rate until the nitroglycerin concentration in the

effluent approached the original nitroglycerin concentration in the bottle. It can be calculated from the data in Figure 5.17 that approximately 350 to 400ml of solution containing nitroglycerin should be flushed through the tubing over several hours to adequately pretreat the tubing before an infusion of nitroglycerin.

(b) Isosorbide Dinitrate

When isosorbide dinitrate solutions were infused from glass infusion bottles through plastic infusion tubing at 0.73 ml/min it was about 4hrs before the effluent concentration of isosorbide dinitrate approached the initial concentration (Figure 5.19c). The loss of isosorbide dinitrate at this flow rate is less than the loss of nitroglycerin solution infused through tubing at a similar flow rate (Figure 5.17). A reduced loss of isosorbide dinitrate is consistent with this drug's lower affinity for the polyvinyl chloride tubing as reflected by polyvinyl chloride-water partition coefficients (Table 5.5).

6.4.3. Role of Infusion Set Components

(a) Nitroglycerin

Adding the plastic infusion bag (Viaflex) and burette (Buretrol) to the infusion tubing reduces the concentration of nitroglycerin appearing in the effluent of the tubing

(Figure 5.18a). The corresponding amount sorbed by the plastic infusion system (Figure 5.18b) for various times of infusion is increased by the addition of these components. During the initial stages of infusion, the concentration of nitroglycerin in the effluent diminishes primarily as a result of sorption by the infusion tubing. At longer times, sorption of nitroglycerin by the plastic burette and infusion bag becomes more significant (Figure 5.18), consistent with the slower process of sorption by the burette and infusion bag compared to the infusion tubing. The apparent steady-state concentration of nitroglycerin in the effluent is less than the original nitroglycerin concentration in the plastic infusion bag/glass infusion bottle as a result of the additional sorption by the infusion bag and the burette.

(b) Isosorbide Dinitrate

When the plastic infusion bag and burette were added to the infusion tubing, the concentration of isosorbide dinitrate in the effluent of the tubing was decreased (Figure 5.19). The greater loss associated with using a giving set to infuse isosorbide dinitrate is due to the drug being lost relatively rapidly when its solutions are stored in the cellulose propionate burettes of the giving sets (Figure 5.10). When using a plastic bag/giving set system the loss is even greater due to the sorption by the plastic bag (Figure 5.19a).

6.4.4. Other Drugs

(a) Diazepam

Literature reports about the possible flow rate dependent loss of diazepam when infusing this drug through plastic giving sets have been conflicting. There was no difference in the concentration of diazepam in the effluent of the giving set tubing when diazepam was infused at flow rates of 24 and 48 ml/hr in one report (Parker and MacCara, 1980) however, profiles of drug loss with time were markedly different when diazepam solutions were infused at rates of 5 ml/hr (MacKichan et al, 1980) and 100 ml/hr (Dasta et al, 1980). The recovery of diazepam after the slower infusion (MacKichan et al, 1980) was about 9%, compared to a recovery of about 64% after an infusion at 100 ml/hr for 5 hours (Dasta et al, 1980).

These results suggest that the loss of diazepam when infused through plastic giving sets is, in part, flow rate dependent just as the losses of nitroglycerin and isosorbide dinitrate are in part due to the flow rate of drug solution through the giving set.

(b) Chlormethiazole

Tsuei et al (1980) reported a flow rate-related loss of chlormethiazole in simulated infusions using plastic giving sets. They found that the greatest loss of chlormethiazole occurred when its solutions were allowed to stand in the giving sets and this loss was progressively reduced as the flow rate was increased from 75 ml/hr up to 999 ml/hr.

6.5. THERAPEUTIC AND PHARMACOKINETIC IMPLICATIONS

6.5.1. Therapeutics

The clinically relevant consequence of drug-plastic interactions involving sorption is that the amount of drug being delivered to the patient is lower than anticipated. For drugs which are used in acute stages of coronary disease it is important that the amount of a drug administered is sufficient and quantified. Although it is still unclear what dose of nitroglycerin is needed to minimize the extent and severity of a myocardial infarction, it has been clearly shown (Chiche et al, 1979; Epstein et al, 1975) that nitroglycerin infusion reduces electrocardiographic signs of extension of necrosis and mortality when associated with left ventricular failure during myocardial infarction.

When nitroglycerin is administered intravenously,

clinicians may use haemodynamic signs, such as reduction in blood pressure or increase in pulse rate, to determine the efficacy of this dosage route. With a fast-acting drug like nitroglycerin it may therefore be possible to titrate the patient to a pharmacological endpoint without the need to know exactly how much drug is being infused. This titration technique may not always be successful. The current work was initiated when patients suffering acute myocardial infarction at the Royal Hobart Hospital failed to register pharmacological endpoints, as measured by changes in some haemodynamic parameters, during infusions of nitroglycerin of amounts supposedly up to 300 mcg/min. Thus this titration technique may not always be successful.

6.5.2. Pharmacokinetics

Some workers have reported the pharmacokinetics of isosorbide dinitrate after oral and sublingual doses (Assinder et al, 1977; Sporl-Radun et al, 1980) but the pharmacokinetics of isosorbide dinitrate after intravenous administration has been published only recently (Taylor et al, 1980). It is essential in clinical pharmacokinetic studies to have an accurate measure of the rate of infusion of a drug. If a plastic bag/giving set system is used to infuse isosorbide dinitrate, blood clearance could be over-estimated by as much as 40% and systemic availability similarly under-estimated. In the report on the pharmacokinetics of isosorbide dinitrate after intravenous

infusion (Taylor et al, 1980) an "infusion set" was used. The nature of the infusion system used by these workers is unclear. If the plastic bag/giving set system described in the current work had been used at an infusion rate employed in the pharmacokinetic evaluation (Taylor et al, 1980) i.e. 1.6 ml/min, a loss of about 20% of initial drug concentration would have occurred as a result of sorption of isosorbide dinitrate by the intravenous administration system. Consequently, systemic availability and volume of distribution would be under-estimated and clearance over-estimated by about 20% respectively.

The detailed pharmacokinetics of nitroglycerin after intravenous infusion have not yet been described. However, Wei and Reid (1979) measured plasma levels of the drug after a slow intravenous infusion. It is not clear what type of intravenous infusion equipment these workers used. In that study there was no correlation between the rate of nitroglycerin infusion and the plasma nitroglycerin concentration. This may have been due to the rapid rate of metabolism of nitroglycerin in the patients or to the reduced availability of nitroglycerin if plastic infusion equipment was used.

6.6. PREVENTION OF LOSS

6.6.1. Organic Nitrates

Losses of nitroglycerin were minimized when solutions of the drug were infused from a glass syringe via a short piece of high density polyethylene tubing. The loss using this system ranged from less than 1% to less than 5% for infusion rates of 0.75 ml/min and 0.07 ml/min respectively (Table 5.3). This reduced loss when compared to the losses recorded when polyvinyl chloride infusion equipment is used is consistent with the very much smaller affinity of nitroglycerin for high density polyethylene as expressed by the plastic-water partition coefficients (Table 5.5).

Mathot et al (1980) have shown that as well as high density polyethylene, teflon and polypropylene can be used to infuse nitroglycerin without loss of the drug. They observed no loss of nitroglycerin when aqueous solutions of it were infused from a polypropylene syringe through polyethylene tubing.

Amann et al (1980) used a polyolefin bottle to prepare nitroglycerin solutions and found that after 48hrs nitroglycerin potencies remained within 6.5% of their initial values. If these bottles were to be used for nitroglycerin infusions, the large loss of nitroglycerin which occurs during transit of the drug solution through

Table 6.3. Recoveries of Diazepam and Chlormethiazole during infusions using glass bottle/plastic infusion sets and glass syringe/infusion pumps.

Drug	Infusion Time (Hours)	Infusion Rate (ml/min)	Per Cent Drug Recovered	
			Plastic Infusion Sets	Glass Syringe Infusion Pump
Diazepam	24	0.08	9.1[a]	100
Chlormethiazole	5	1.1	78.2[b]	100

[a] MacKichan et al (1979) (estimated from area under curve)

[b] Tsuei et al (1980).

plastic giving sets would still have to be overcome.

Isosorbide dinitrate is not lost when its aqueous solutions are infused from glass syringes through high density polyethylene tubing (Table 5.3). This finding is in accordance with the almost negligible partitioning of isosorbide dinitrate between water and high density polyethylene (Table 5.5).

6.6.2. Other Drugs

The glass syringe-high density polyethylene tubing infusion system also prevents the previously reported loss of chlormethiazole (Tsuei et al, 1980) and diazepam (MacKichan et al, 1979) when used at comparable infusion rates to those used in their studies, during infusion of these drugs through plastic giving sets (Table 6.3).

6.7. FUTURE WORK

The results presented in the current work show that patients receiving intravenous infusions of nitroglycerin or isosorbide dinitrate via plastic intravenous infusion equipment could receive only a small fraction of the intended doses. This example shows that a drug-plastic interaction has created the potential for therapy to fail. Other drugs need to be evaluated in terms of their ability to be sorbed by any plastic container with which they come

into contact. Moreover, new plastic containers should be evaluated for possible leaching of toxic substances from them into drug solutions. These areas of investigation are relatively more important for drugs and plastics associated with parenteral therapy. Recently Kowaluk et al (1981) surveyed a large number of drugs currently administered parenterally in order to detect drugs which are liable to be affected by drug-plastic interactions and this sort of survey may have to be done periodically to evaluate possible interactions between new drugs and their plastic delivery systems.

Further work on the most appropriate models to mathematically describe the loss of solutes from solutions stored in plastic containers is required if the dynamics of drug-plastic interactions are to be better understood. The processes which control the disappearance of solutes have not been fully described and so work in this area could proceed with a view to gaining some predictive index of interaction.

Evaluation of the compatibility of existing drugs with plastic delivery systems and the ability to predict such interactions for new drugs should minimize potential drug-plastic interactions and possible therapeutic failure resulting from those interactions.

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